DEVELOPMENTAL STUDIES OF CEFAZOLIN LOADED BOVINE SERUM ALBUMIN NANOPARTICLES

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CERTIFICATES

بسم الله الرحمن الرحيم
In the Name of Allāh, the Most Gracious, the Most Merciful

Prof. Dr. A. ABDUL HASAN SATHALI, M.Pharm., Ph.D,
Principal (i/c),
College of Pharmacy,
Madurai Medical College,
Madurai-625 020. (TN), India.
Email: dr*abdulhasan@rediffmail.com*
Mob: 09443475400

Res: 19, Nallamani Nagar,
Lordu Nagar 12th Street,
K-Pudur,
Madurai – 625007. (TN), India.

CERTIFICATE

This is to certify that the dissertation entitled **"Developmental studies of Cefazolin loaded Bovine serum albumin nanoparticles"** submitted by **Mr. C. Pravinkumar (M.Pharm II year)**, in partial fulfillment of the requirement for the Degree of **Master of Pharmacy** in **Pharmaceutics**, is a bonafide work carried out by him, under my guidance and supervision in the Department of Pharmaceutics, College of Pharmacy, Madurai Medical College, Madurai – 20 during the academic year 2013 – 2014.

This dissertation is forwarded to the Controller of Examinations, The Tamilnadu Dr. M.G.R. Medical University, Chennai-32.

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(Prof. Dr. A. ABDUL HASAN SATHALI)

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(PRAVINKUMAR.C)

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CHAPTER I

INTRODUCTION

CHAPTER - I

INTRODUCTION

Nanotechnology can be defined as the science and engineering involved in the design, synthesis, characterization and application of materials and devices, whose smallest functional organization in atleast one dimension is on the nanometer scale (one billionth of a meter). It can prove to be a boon for human health care. Because nano science and nanotechnology have a huge potential to bring benefits in areas as diverse as drug development, water decontamination , information and communication technologies and production of stronger /lighter materials. Human health nanotechnology research can definitely result in immense health benefits. The genesis of nanotechnology can be traced to the promise of revolutionary advances across medicines, communications, genomics and robotics(SahooS.K. et al., 2007). Different types of nano sized carriers, such as polymeric nanoparticles, solid lipid nanoparticle , ceramic nanoparticles, magnetic nanoparticles, polymeric micelles, polymer-drug conjugates, nanotubes, nanowires, nanocages and dentrimersetc, are being developed for various drug delivery (Sahoo S.K. et al., 2008). Polymeric nanoparticles can be fabricated from polysaccharides, proteins and synthetic polymers. Nanoparticles made from natural hydrophilic polymershave been proved efficient interms of better drug loading capacity, bio compatibility and possibly less opsonization by reticuloendothelial system through an aqueous steric barrier(Liu Z. et al., 2008).

Nano antibiotics:

The bacterial resistance to antimicrobial drugs has been attempted to be resolved by discovery of new antibiotics unfortunately there is no assurance . One of the recent efforts in addressing this challenge lies in exploring antimicrobial nanomaterials, to which microbial pathogens may not be able to develop resistance and novel nanosized platforms for efficient antibiotic delivery.Antimicrobial nanoparticles offer many distinctive advantages in reducing acute toxicity, overcoming resistance and lowering cost when compared to conventional antibiotics. Theoretically nanoparticles are retained much longer in the body than small molecule antibiotics, which could be beneficial for achieving sustained therapeutic effects. Use of nanotechnology in immunization, design and delivery of antimicrobial drugs, diagnosis and control of cross infections in particular overcoming antibiotics resistant pathogens alternative to the current antibiotics based approaches.

Nanomaterial, which either show antimicrobial activity by themselves or elevate the effectiveness and safety of antibiotics administration, are called **"nano antibiotics"** and their capability of controlling infections invitro and in vivo has been explored and demonstrated.

Recently, antibiotics formulated in polymeric nanoparticles have demonstrated enhanced antimicrobial activities and anti MRSA activities, compared with non polymerized forms of penicillin and N- methylthio β lactams. A wide range of antimicrobial agents can be effectively administered using various nanoparticles. Many types of liphophilic and water soluble antibiotics can be conjugated inside or on the surface of nanoparticles or carried via encapsulation. Pharmacokinetics characteristics of antibiotics, including improved solubility, controlled release and

specific site targeted delivery can be achieved by employing appropriate nanocarriers.

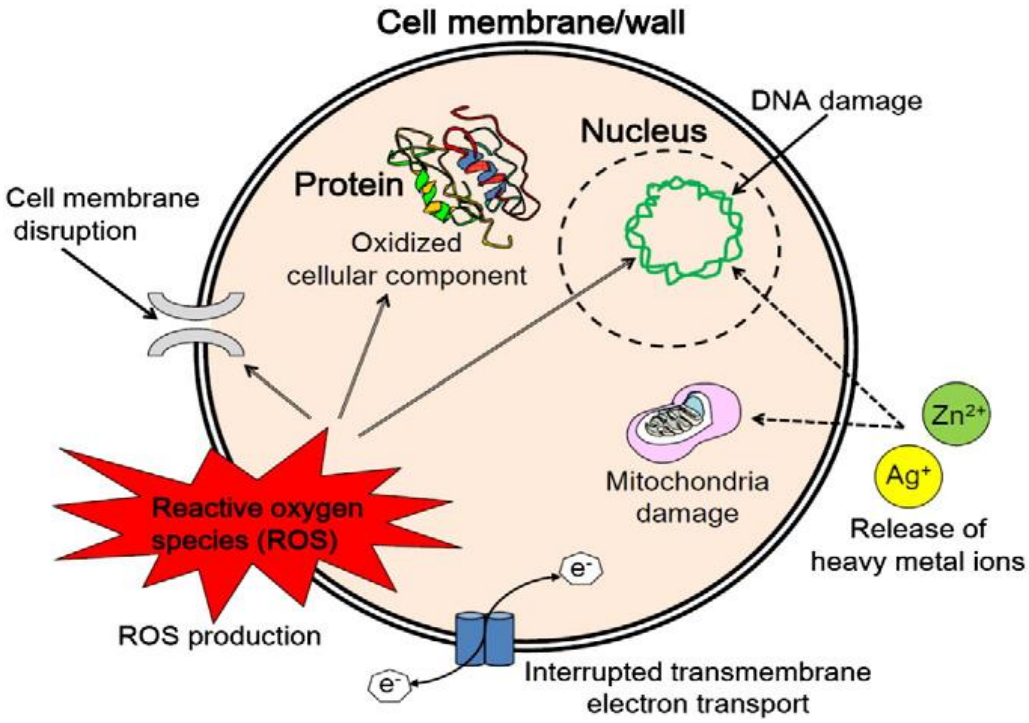


FIGURE (1).VARIUOS ANTIMICROBIAL MECHANISMS OF NANOMATERIALS.

Advantages of nanoantibiotics:

- ❖ They are stable enough for long-term storage with a prolonged shelf-life.
- ❖ Controllable and relatively uniform distribution in the target tissue.
- ❖ Improved solubility.
- ❖ Sustained and controlled release.
- ❖ Improved patient compliance.
- ❖ Minimized side effects.

- Enhanced cellular internalization.
- Prolonged drug half-life and systemic circulation time.
- Sustained and stimuli-responsive drug release.
- Eventually lowers administration frequency and dose.

(Young Jik Kwon et al., 2011)

Antimicrobial Polymeric nanoparticles:

The first polymer based delivery of macromolecules was demonstrated in 1976, controlled drug release using biocompatible and biodegradable polymers further emerged in the 1980's and has been extensively investigated in the clinic for enhanced intracellular drug delivery and reduced rapid clearance by reticuloendothelial system.

There are currently two major types of polymericnanoparticles for antimicrobial drug delivery. One is formed*via* spontaneous self-assembly of diblock copolymers consistingof hydrophilic and hydrophobic segments. The hydrophobicsegment forms a polymeric core containing thedrugs while the hydrophilic segment shields the core from

opsonization and degradation. The rate of drug release canbe tuned by varying the length of the hydrophobic chain. Avariety of biodegradable polymers have been used to formthe hydrophobic polymeric core, including poly(lactic acid)(PLA), poly(glycolic acid) (PGA), poly(lactide-co-glycolide)(PLGA), poly (□-carprolactone) (PCL), and poly(cyanoacrylate) (PCA),whereas polyethylene glycol (PEG) hasbeen commonly used as a hydrophilic segment. Diblock copolymernanoparticles are typically prepared through solventdisplacement. In this process, polymers and drugs are firstdissolved in a water-miscible organic solvent such as acetonitrile.The

polymer-drug mixture is then added to an aqueoussolution. As the organic solvent evaporates, the blockcopolymers and drugs undergo nanoprecipitation to formnanoparticles consisting of a hydrophobic core and a hydrophilicshell. Polymeric nanoparticles are primarily used tocarry and deliver poorly water soluble drugs because of thehydrophobic nature of the nanoparticle core.

The other type of polymeric nanoparticles consists of linearpolymers such as polyalkyl acrylates and polymethylmethacrylate that form nanocapsules through an emulsionpolymerization method. In this process, monomers are firstdissolved in polymerization media in the presence of surfactants.Polymerization initiators are then added to the solutionto trigger polymerization resulting in the formation of nanocapsules.Antimicrobial drugs can be either absorbed to thenanocapsules during the polymerization process or covalentlyconjugated to the surface of the nanoparticles afterthey are formed. The absorption process favors hydrophobicdrugs as it requires dissolving the drugs to an oil phase. Hydrophilicdrugs are usually attached to the particle throughcovalent conjugations. It is worth noting that in the case ofcovalent linkage, antimicrobials can be inactivated and needto be verified of their activity before use. For instance, in astudy on treating *staphylococcal* infections, Abeylath*et al*.have observed that β-lactam and ciprofloxacin retained theirpotency whereas penicillin was inactivated upon covalentattachment to nanoparticles .Polymeric nanoparticles have been explored to deliver avariety of antimicrobial agents to treat various infectiousdiseases and have shown great therapeutic efficacy. For example,the antimicrobial activity of amphotericin B-loadedpoly(β-caprolactone) nanospheres coated with non ionic surfactantpoloxamer 188 have shown greater therapeutic efficacyagainst *LeishmaniaDonovani*has compared to the freedrug counterparts. Rifampicin-loaded polybutylcyanoacrylatenanoparticles have shown enhanced

antibacterialactivity both *in vitro* and *in vivo* against *Staphylococcusaureus*and *Mycobacterium avium*due to an effective delivery of drugs to macrophages(Zhang L. et al., 2010).

Proteins are class of natural molecules that have unique functionalities and potential applications in both biological as well as material fields. Controlled drug release and subsequent biodegradation are important for developing successful formulations(Karthick Raja NamasivayamS. et al., 2013). System based on proteins including gelatin, collagen, casein, albumin and whey protein have been studied for delivering drug, nutrients, bioactive peptides and probiotic organisms. Proteins represent good raw materials, since they have the advantages of absorbability and low toxicity of the degradation end products.

Potential release mechanisms involve in protein nanoparticles are

- Desorption of surface – bound / adsorbed drugs.
- Diffusion through the carrier matrix.
- Diffusion through the carrier wall (in the case of nanocapsules).
- Carrier matrix erosion/diffusion process.

(Karthick Raja Namasivayam S. et al., 2013).

Albumin nanoparticles:

Among the available potential colloidal drug carrier systems, protein based nanoparticles are particularly interesting as they hold certain advantages such as greater stability during storage nontoxic, non antigenic and their ease to scale up during manufacture over other drug delivery systems. Albumin based nanoparticle

carrier system represents an attractive strategy, since a significant amount of drug can be incorporated into the particle matrix because of the different drug binding sites present in the albumin molecules. Due to the defined albumin primary structure and high content of charged amino acids. Albumin based nanoparticles could allow the electrostatic adsorption of positively or negatively charged molecules without the requirement of other compounds. In addition, albumin nanoparticles can be easily prepared under soft condition by coacervation, controlled desolvation or emulsion formation. They show smaller particle size compared to microparticle, better controlled release properties than liposomes which may improve patient acceptance and compliance(Ahmed O.Elzoghby et al., 2012).

Advantages:

Albumin is an attractive macromolecular carrier that has been shown to be biodegradable, non toxic, metabolized invivo to produce innocuous degradation products, non immunogenic, easy to purify and soluble in water, allowing easy of delivery by injection. Nanoparticle made of albumin are biodegradable, easy to prepare and reproducible. Due to the high protein binding of various drugs, the matrix of albumin nanoparticle can be used for effective incorporation of these compounds. Covalent derivatization of albumin nanoparticles with drug targeting ligands is possible, due to the functional groups on the nanoparticles surface.

Preparation techniques of albumin nanoparticles

- Desolvation method (coacervation)
- Emulsification method
- Thermal gelation method
- Nano spray drying method

- ❖ Nab – technology (nanoparticle albumin bound technology)
- ❖ Self assembly method

Desolvation method:

In desolvation process, nanoparticles are obtained by continuous dropwise addition of ethanol to an aqueous solution of albumin under continuous stirring until the solution became turbid. Then coacervates were hardened by crosslinking with glutaraldehyde.er ethanol elimination by evaporation under reduced pressure. Prepared nanoparticles were purified by centrifugation to eliminate the free albumin and the excess crosslinking agent. The nano suspension was freeze dried using 5% mannitol as a cryoprotectant to obtain a fine powder of the nanoparticles. Desolvation method is a robust and reproducible method for the denaturation of albumin.

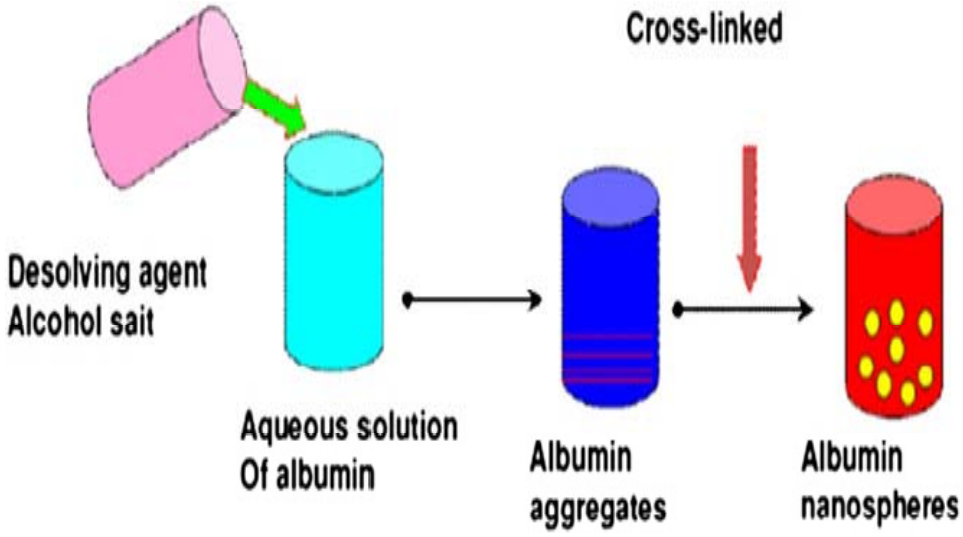


FIGURE (2). PREPARATION OF ALBUMIN NANOPARTICLES BY DESOLVATION METHOD

Emulsification method:

Emulsification technique has been extensively used for preparation of polymeric nanoparticles. Two main methods are used for stabilization of albumin nanoparticles, thermal or chemical treatment. Albumin nanospheres of 0.3 - 1µm were formed by homogenizing the oil phase containing the albumin droplets at a high speed. Then thermally stabilized by heating at 175º to 180ºc for 10 minutes. This mixture was cooled and diluted with ethylether to reduce the oil viscosity to facilitate separation by centrifugation. In chemical stabilization albumin aqueous solution was emulsified in oil at 25ºc then denatured by resuspension in ether containing the cross linking agent.

This technique requires organic solvents for removal of both oily residues and surfactants. Furthermore thermal stabilization is applicable only to drug molecule that are not heat sensitive. However chemical stabilization, the toxicity of the chemical crosslinking agents remain the main problem.

Thermal gelation:

Thermal gelation is a sequential process , that involves heat induced unfolding followed by protein-protein interactions including hydrogen bonding, electrostatic, hydrophobic interactions and disulfide-sulfhydryl interchange reactions. Thermal gelation is a successful method to prepare albumin nanoscale hydrogel for loading and releasing drugs.

Self assembly:

In this method increasing the hydrophobicity of albumin by addition of liphophilic drug and diminishment of primary amine groups on protein surface could

drive the self assembly of HSA and formation of polymeric micelles. Nanoscale micelles prepared in this method can provide high deree of drug loading at the hydrophobic core of micelles acts as a micro reservoir for poorly water soluble drugs.

Nano spray drying:

Spray drying is a well estabilished method commonly used in the pharmaceutical industry for preceding a dry powder from a liquid phase. Unlike conventional spray dryer, the newnano spray drier utilizes a vibratingmesh technology for fine droplets generation. Basically the piezoelectric crystal driven spray head is incorporated with a small spray cap that contains a thin perforated membrane (spray mesh) having an array of precise micron seized holes. When the piezoelectric activation is driven at an ultrasonic frequency (i.e60 KHz), the mesh will vibrate upwards and downwards, injecting millions of precisely sized droplets from the holes and generating the aerosols.

In contrasts to common cyclone technology, particle separation in the nano spray dryer involves the use of the electrostatic precipitator where by the collection mechanism is independent of particle mass. Collection of fine particles with high efficiency is achieved with the novel electrostatic particle collector consisting of a grounded star electrode (cathode), and cylindrical particle collecting electrode(anode). The presence of high voltage around the particle collector creates an electrostatic field that accelerates the deposition of negatively charged particles onto the inner wall of particle collecting process. This is followed by a discharging process **(**Lee S.H. et al., 2011)

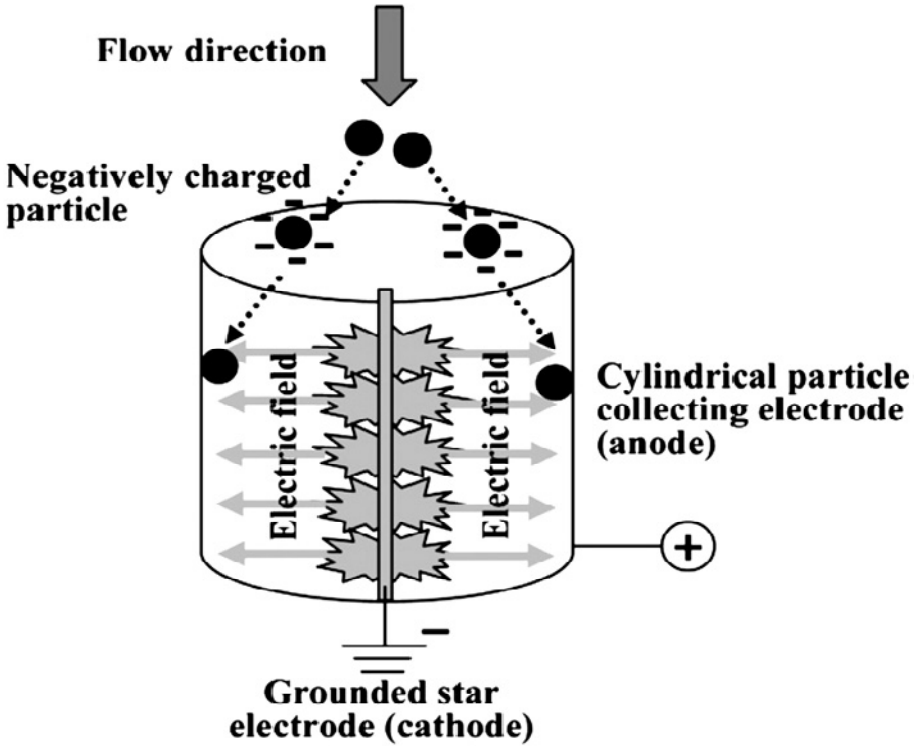


FIGURE (3).THE FUNCTIONAL PRINCIPLE OF AN ELECTROSTATIC PARTICLE COLLECTOR IN THE NANO SPRAY DRYER.

It has advantage of drying and particle formation in a single step continuous and scalable process.

Nanoparticle albumin bound technology:

American bio science inc, has developed a unique albumin bound nanoparticle technology (nab-technology) that is ideal for encapsulating liphophilic drug into nanoparticles. The drug is mixed with HSA in an aqueous solvent and passed under high pressure through a jet to form drug albumin nanoparticles in the size range of 100-200nm. Several nab drugs are currently under development.(Desai N.abraxis bioscience inc, 2007).

Nab technology were designed to be safe and suitable for intravenous usage of poorly soluble drugs. Albumin bound nanoparticle technology does not require surfactants or polymeric materials for preparation. In this method sulfhydryl residues of HSA may be oxidized (and existing disulfide bonds may be disrupted) to form new crosslinking disulfide bonds. However, the disulfide formation induced by homogenization does not substantially denature the HSA.

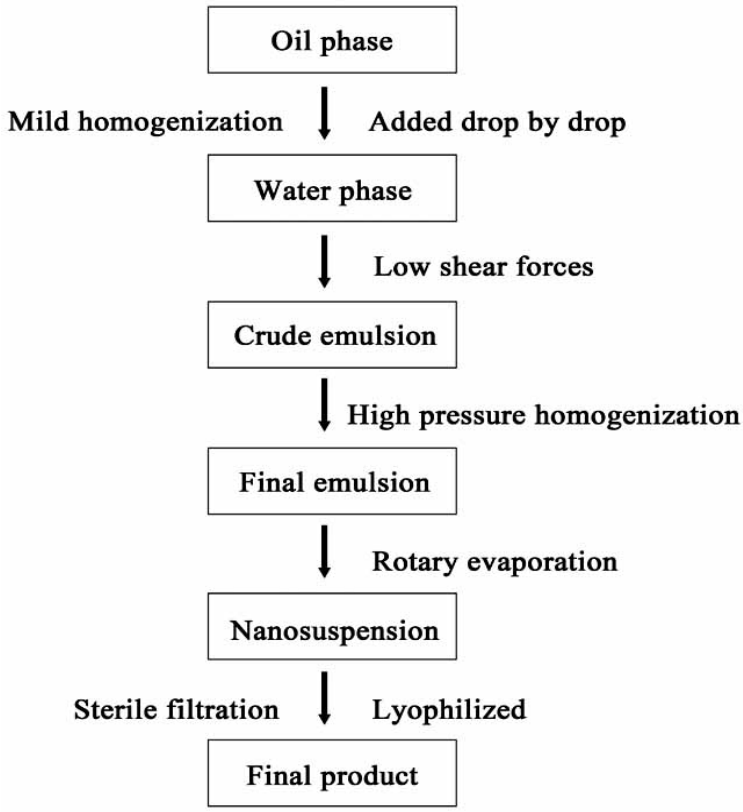


FIGURE (4). PREPARATION OF ALBUMIN NANOPARTICLES BY NANOPARTICLE ALBUMIN BOUND TECHNOLOGY.

Surface modified albumin nanoparticles:

Albumin based nanoparticle offer various possibilities for surface modification due to the presence of functional groups (i.e. carboxylic and amino group). On the surface of the nanoparticles conjugation of surface modifying ligands

is usually achieved through covalent bond formation between the ligands and the functional groups of albumin surface. However surface coating or electrostatic adsorption techniques may be also utilized for surface modification of the nanoparticles. In the albumin – ligand combinations the protein acts as a biodegradable carrier for drug delivery where as ligand is used for,

- Modification of pharmacokinetic parameters (e.g. surfactants)
- Enhancing the stability (e.g. poly-L-lysine)
- Prolonging its circulation (e.g. poly ethylene glycol)
- Slowing the drug release (e.g. cationic polymer)
- Targeting agent (e.g. folate, transferrin, apolipoptrotein and monoclonal antibodies)

(Ahmed O.Elzoghby et al., 2012).

CHAPTER II

LITERATURE REVIEW

CHAPTER – II

LITERATURE REVIEW

Abdul HasanSathali.A*et al.*, 2011, studied to improve the ocular bioavailability of levofloxacin and also reducing the frequency of administration. Niosomalinsitu gelling system was selected to provide prolonged action by preventing the enzymatic metabolism of the drug. Noisomes are formulated by thin film hydration method. pH triggered gelling system prepared using polymers such as carbopol -940 and HPMC K4M, formulation were evaluated for their clarity, pH, antimicrobial activities.

Ahmed O.Elzoghly*et al.*, 2012, discussed about albumin nanoparticles with respect to types, formulation aspects, major outcomes of invitro and invivo investigation, as well as site specific drug targeting using various ligands modifying the surface of albumin nanoparticles with special insights to the field of oncology. And also discussed about specialized nanotechnological techniques like desolvation, emulsification method, thermal gelation, recently nano spray drying, nab technology and self assembly method.

Amit das *et al.*, 2004, investigated the structural changes of bovine serum albumin during its unfolding in the presence of urea, a small angle neutron scattering study was performed. The scaterring curves of dilute solution of BSA with different concentrations of urea in D_2O at pH 7.2 ± 0.2 were measured at room temperature, scattering profile was fitted to a prolate ellipsoidal shape of the protein with a=52.2 A° and b=24.2 A° . thedimentions of the protein as it unfold was found to be anisotropic.

AnkaraoA. *et al.,* 2012, prepared the oral sustained release nanoparticles of carvedilol, nanoparticles were prepared by coacervation method using ethanolicglutaraldehyde as crosslinking agent. The particle size was ranging from 500-1000nm, the maximum drug entrapment and yield were about 76.82 and 76.55 respectively. The sustained release behavior of nanoparticles evaluated both acid and phosphate buffer.

Bigucci F.*et al.,* 2011, fabricated albumin nanoparticles carrying cyclodextrins for nasal delivery of tacrine prepared by coacervation method followed by thermal crosslinking. The drug was loaded by soaking the prepared nanoparticles in a solution of tacrine hydrochloride then lyophilized. Thermal analysis and FTIR were performed in order to confirm protein cross linking and possible drug carrier interaction. Prepared nanoparticles were evaluated for particle size, zeta potential and morphology of surface, as well as drug loading, mucoadhesion properties and ex vivo drug permeation ability.

EspuelasmillanM.S. *et al.,* 2004, evaluated the potential of albuminnano particles as a delivery system for antisense oligonucleotide. Nano particles were prepared by a coacervation process and cross-linkage with glutaraldehyde. Phosphodiester(PO) and phosphorotiate (PS) oligonucleotide were either adsorbed on the surface of nano particles (PO-NPA and PS-NPA) or incorporated in the nano particles matrix (PO-NPB and PS-NPB). The antiviral activity was evaluated in MRC-5 fibroblasts infected with human cytomegalovirus at a MOI of 0.0035. Both PO nano particles formulation significantly increased the antiviral activity, these finding were well correlated with the intracellular distribution observed for fluorescent oligonucleotide-loaded albumin nano particles. Albumin nanoparticles

partially protected a PO against enzymatic degradation and improved their presence in the nucleus.

Fabio bignotti*et al.,* 1997, prepared surface modified albumin nanospheres with a size of around 100nm in diameter from poly(amidoamine)- poly (ethyleneglycol) copolymer grafted human serumalbumin and poly(thioetheramido acid) – poly (ethylene glycol) copolymer grafted human serum albumin. The nanospheres were produced using pH coacervation method and cross linked with glutaraldehyde. The cross linking efficiency was affected by the type of albumin conjugate used, zeta potentials of the surface modified nanospheres was significantly lower than that of unmodified particles, and also reduced plasma protein adsorption on the particle surface.

Frangworek*et al.,* 2011, developed HI6 human serum albumin nano particles to deliver variety of drugs across the BBB into the brain. HI6 dimethanesulfonate and HI6 dichloro monohydrate were bound to nano particles. At the pH of 8.3 maximum drugs binding capacity of 344.8 µg and 322.6 µg per mg of nano particles were calculated for HI6 dimethanesulfonate and HI6 dicholoro monohydrate respectively. Invitro testing of the nano particulate oxime formulations in primary porcine capillary endothelial cells demonstrated an upto two times higher reactivation of organophosphorus inhibited aceylcholinestrase than the free oximes. These finding shows that nano particles made of HSA may enable a sufficient antidote for organophosphorus poisoning therapy with HI6 derivatives even within the central nervous system.

Guanghui ma *et al.,* 2008, developed thermosensitivepoly(N-iso propyl acryamide-co-acrylamine) conjugated albumin nanospheres for thermally

targeting the anticancer drugs. Albumin nanoparticles with below 200nm and narrow size distribution was successfully prepared in the first step with desolvation technique. Then in the second step the polymer was conjugated on to the surface of the albumin nanoparticles by rdical polymerization. Adriamycin was then entrapped into the albumin nanoparticle and polymer conjugated albumin nanoparticle(PAN) during the particle preparation. Compared with albumin nanoparticle, the drug release from PAN in trypsin solution was slower.

Guido barone*et al.,* 1997, analysed the thermal denaturation process of defatted bovine serum albumin. DSC measurements were performed on changing the pH , ionic strength and the sodium dodecyl sulphate concentration. Measurements shown that the thermal denaturation is always irreversible regardless of pH, ionic strength and SDS concentration. The strong stabilizing effect of Nacl on the BSA native structure has been found for the range 0-1M, sodium dodecyl sulfate has a net stabilizing effect up to a molar ratio of 10:1.

Hasanuluda*get al.,* 2008, investigated the preparation process of bone morphogenetic protein 2 containing bovine serum albumin nanoparticles. The nanoparticles were prepared by a coacervation method. Polymer coated nanoparticles were characterized with respect to amount of adsorbed polymer , particle size and zeta potential. Using bone marrow stromal cells biocompatibility of the nanoparticles was investigated by the MTT assay. The size of the nanoparticles could be controlled in the range of 50-400nm by process parameters, particle size and zeta potentials were increased after coating with cationic polymers.

Hiroshi saitoh*et al.,* 2002, investigated the correlation between invitro permeation of 11 β-lactam antibiotics across rat jejunum and their oral bioavailability in humans. The absorptive and secretory permeation across the rat jejunum was evaluated. A steep sigmoid curve was obtained for the relationship between P_{app} in the absorptive permeation and human oral bioavailability , the ratios of P_{app} in the absorptive direction to P_{app} in the secretory direction were plotted against human oral bioavailability , a much improved correlation was obtained ($r = 0.98$, $p<0.001$).

Irache J. M. *et al.,* 2001, studied the invitro drug release properties of the three different formulations of gemcitabine loaded albumin nanoparticles prepared by coacervation method. Formulation were differentiated depending on the steps where the drug and/or cross linking agent added. In all formulation particle size ranges between 200-400nm, yield 50%in model A, 65%in model B. nanoparticle shows biphasic release profiles. Burst release was especially relevant in model A(60%in 1 hour), model B (40%in 1 hour), model C (20% in 1 hour).

IracheJ.M. *et al.,* 2002, formulated albumin nano particles as carrier for a phosphodiester oligonucleotide. Bovine serum albumin nano particles were formulated by coacervation process. A phosphodiester oligonucleotide incorporated by incubation with the albumin prior to the coacervation process or adsorbed onto the prformednano particles. Incorporated and/or adsorbed oligonucleotide was estimated by capillary electrophoresis and fluorescence spectroscopy. The adsorption of oligonucleotide was dependent on the pH of the medium. Desorption of the oligonucleotide also affected by the pH and ionic strength of the medium. Oligonucleotide incubated with the albumin prior to nano particle formation , the profile of release confirmed that a fraction was

incorporated into the matrix and its release was controlled by the albumin degradation.

Isabelle Migneault*et al.*, 2004, reviewed glutaraldehyde behavior in aqueous solution, reactions with proteins and application to enzyme crosslinking. Glutaraldehyde one of the most effective protein crosslinking reagents, it has atleast 13 different forms depending on solution conditions such as pH , concentration, temperature etc. Glutaraldehyde may react with proteins by several means such as Aldol condensation or Micheal type addition. Glutaraldehyde can react with several functional groups of protein such as amine, thiol, phenol and imidazole because the most reactive aminoacid side-chains are nucleophiles. No single mechanism seems to be responsible for glutaraldehydecrooslinking with proteins.

Jahanshahi M.*et al.*, 2008, optimized the formulation of bovine serum albumin nanoparticles by applying Taguchi method. Agitation speed, initial BSA concentration , pH and temperature were considered as process parametersthe result indicates temperature and agitation speed were the most influencing parameters on the particle size. The minimum size of nanoparticles (74nm) obtained at 4ºc, pH 7.5, 15mg/ml BSA concentration and agitation speed of 500rpm.

JahanshahiM. *et al.*, 2006, fabricated bovine serum albumin nanoparticles, several process parameters examined to achieve suitable size. The smallest size of nanoparticle was 101nm and the largest size was 503 nm. Agitation speed , temperature of the mediumwere the most effective parameters. The minimum size of nanoparticlewas obtained at 300-400rpm and 30mg/ml protein concentration.

The nanoparticles sample was purified with 50000g centrifugation then followed by dialysis, micro and ultra filtration then analyzed.

Kang Choon Lee *et al.,* 2011, prepared curcumin loaded human serum albumin nanoparticles for intravenous administration using albumin bound technology. Particle size in the range of 130-150nm, it was investigated by field emission scanning electron microscopy and dynamic light scattering and also investigate the water solubility of curcumin (300 fold increased). The amount of curcumin in tumor after treatment with drug loaded nanoparticles shows 14times higher than that of curcumin at 1 hour.

Kavitadua*et al.,* 2013, formulated didanosine loaded bsa nanoparticles by desolvation techniques, and optimizes the formulation by selecting four process variables, stirring speed , pH of the solution , polymer concentration, amount of cross linking agent. Nanoparticles prepared at stirring speed 700rpm, pH8.5, polymer concentration 60mg/ml and amount of glutaraldehyde 0.6µl/mg of bsa was considered as optimized formulation.

LangerK. *et al.,* 2003, developed desolvation procedure for the preparation of HSA-based nanoparticles between the size range of 100nm-300nm. A pump controlledformulation method was established which enabled particle preparation under defined conditions. Several factors of the preparation process were evaluated. The major factor to determining the particle size is pH of the HSA solution, higher pH leading to smaller particle size, washing the particles by differential centrifugation leads to significantly narrowersize distribution. Stability of the resulting nanoparticles was evaluated by pH and buffer titration method.

Only pH values outside the isoelectric pH range of HSA and low salt concentration were able to prevent nanoparticle agglomeration.

Langer K.*et al.*, 2008, systematically evaluated excipients for the freeze drying and long term stability of albumin based nanoparticles. Empty, doxorubicin loaded and PEG-lated nanoparticles were prepared and were freeze dried in the presence of different concentration of sucrose, trehalose and mannitol respectively. The samples were physicochemically characterized with regard to lyophilisate appearance, particle size and polydispersity using photon correlation spectroscopy. For evaluation of long term stability samples were stored at 2-8ºc, 25ºc and 40ºc over predetermined time intervals, particle growth was observed in all freeze dried formulations in the absence of cryoprotectants, sucrose and trehalose were superior to mannitol.

Langer K.*et al.*, 2010, studied about specific transport of anticancer drugs into tumor cells. Expression of αvβ3 integrins holds promise for anticancer therapy. DI17E6 (monoclonal antibody) inhibits growth of melanomas invitro and in vivo, also inhibits angiogenesis due to interference with αvβ3 integrins. DI17E6 covalently coupled to human serum albumin nanoparticles, this nanoparticles specifically targeted αvβ3 integrins positive melanomas.

Langer K.*et al.*, 2000, optimized the desolvation process of Human serum albumin for the preparation of nanoparticles, the resulting nanoparticles were stabilized by the addition of varying amount of glutaraldehyde or by heat denaturation. The nanoparticles evaluated for particle size, zeta potential, and number of amino groups on the surface. The results indicated the particle size depends mainly on the amount of desolvating agent added. Increased amount of

glutaraldehyde decreases amino groups on the surface of HSA and also the zeta potential. Temperature and heat denaturation time influences the stability of HSA nanoparticles.

Langer K.*et al.*, 2007, formulated doxorubicin loaded HSA nanoparticles it may diminishes the toxicity and optimizes the body distribution of drug, may overcome multi drug resistence. Drug was loaded either by adsorption to the nanoparticles surface or by incorporation into the particle matrix. Particle size between the range of 150nm and 500nm with loading efficiency of 70% to 95%. Cell viability ofnanoparticles was investigated in two different neuroblastoma cell lines. Anticancer activity of drug loaded nanoparticles were increased in comparison to doxorubicin solution.

Li F.Q.*et al.*, 2008, prepared sodium ferulate loaded albumin nanoparticles by desolvation crosslinking method for liver targeting. The nanoparticles were spherical in shape, particle size between 100nm and 200nm, charged with zeta potential from -20 to -40 mV. Drug entrapment efficiency was decreased along with increased amount of glutaraldehyde used for cross linking; invitro drug release shows initial burst release and then sustained release.

Lingdongquan*et al.*, 2013, reviewed, albumin as a drug or imaging agent carrier system for rheumatoid arthritis. There are three major types of albumin based carrier system for rheumatoid arthritis, including albumin drug conjugates, albumin particles and genetic infusion albumin. Their imaging or therapeutic effects have been proved in clinical or preclinical studies. The enhanced fenestration allows albumin based delivery systems to extravasate at the inflammation site.

Pedroso T.M *et al.,* 2013, developed UV spectroscopy method validation for quantification of cefazolin sodium in pharmaceutical form powder for injectable solution. The method presented being capable to detect and quantify the drug , and also determined the linear range of concentration, obtaining satisfactory results in the linear range of 8 to 28µg/ ml, showing correlation coefficient of 0.9999 when analyzed in the wavelength of 270nm.

Peppas N.A. *et al.,* 2006, reviewed about opsonization ,biodistribution , and pharmacokinetics of polymeric nanoparticles. Opsonin proteins in serum quickly bind to conventional non stealth nanoparticles , allowing macrophages of MPS to easily recognize and remove these drug delivery devices before they can perform their designed therapeutic function. Pegylation is one of the method to address these limitations, it increases the circulation half life of the nanoparticles. This method creates a hydrophilic protective layer around the nanoparticles, blocking and delaying the adsorption of opsonin protein via steric repulsion forces.

Ping yao*et al.,* 2013, formulated biodegradable doxorubicin loaded bovine serum albumin nanoparticles, with folic acid modified dextrin surface by pH adjustment and heating process. The nanoparticles have a size about 90nm and excellent dispersity at pH 7.4 aqueous solution. The drug loading efficiency is 90%. Anti tumor activity of the nanoparticles are evaluated through murine ascites hepatoma H22 tumor bearing mice.

Rintibanerjee*et al.,* 2012, formulated albumin based nanoparticles for ophthalmologic application of aspirin. By varying aspirin albumin ratios from 0.06 to 1, they obtained, electrokinectically stable, pharmacologically active albumin based aspirin nano particles of < 200nm diameter with low

polydispersity. Invitro release study showed nanoparticles formulation can release aspirin at a sustained rate for prolonged duration (90% at72 hours) and 11% drug release in the posterior chamber over a period of 72 hours under simulated condition. Stability of the formulation was well maintained on storage for six months. The formulation showed no hemolysis in contrast to the free drug.

Sambadananandan*et al.*, 2013, studied the detailed information about the interactions of various drug and nanomaterials with human and bovine serum albumin. The interaction was studied by fluorescence spectroscopy due to its high sensitivity and straight forwardness.

SanghoonKo*et al.*, 2011, formulated bovine serum albumin nanoparticles using modified desolvation method. Bovine serum albumin nanoparticles and calcium loaded bovine serum albumin nanoparticles were fabricated at controlled sizes of 100nm, 400nm, 800nm. Particle size and surface area to volume ratios of the prepared bsa nanoparticles were measured, and it controlled by adjusting bsa concentration, pH, sodium chloride content wich affects the coagulation of the bsa molecules.

Sanjudhawan*et al.*, 2011, prepared bovine serum albumin nanoparticles loaded with paclitaxel using desolvation technique. A 3^2 full factorial design was employed to formulate nanoparticles. Response surface linear modeling was used to predict the optimal formulation. Various models were applied to determine the release mechanism.

Satyaprakash*et al.*, 2010, developed noscopine loaded human serum albumin nanoparticles for targeted drug delivery to tumor cells by enhancing the drugs bioavailability , distribution and reducing the body's response towards drug

resistance. The nanoparticles were designed and optimized to achieve a particle size in the range of 150 – 300 nm with a drug loading efficiency of 85-96%.

Seyedabbasshojaosadati*et al.*, 2008, prepared 5-fluorouracil loaded bovine serum albumin nanoparticles by phase separation method. Drug release was tracked by continuous flow dialysis method. Process variables was optimized by through taghuchi's M16 design. Optimum condition wass found to be 2mg/ml of fluorouracil, 3.7ml of ethanol, 176µg of gluaraldehyde, drug protein incubation time 30minutes and pH of 8.4, mean diameter were 210nm and zeta potential of -31.7mV.

VijayarajkumarP. *et al.*, 2007, prepared HSA – pectin nano particles loaded with ciprofloxacin by coacervation method, pectin used to suppress the agglomeration of ciprofloxacin loaded human serum albumin nano particles. Various physicochemical parameters were evaluated , size was found to be 180-290nm.the critical flocculation concentration of nano particles in Na_2so_4 solution was increased from 0.3-0.9M , isoelectric points of the nano particles were found to be relatively lower between pH values 3 and 6.

Wilson B.*et al.*, 2012, formulated and evaluated nanoparticles based on albumin to deliver 5-fluorouracil, prepared by coacervation method. The drug loading capacity varied from 4.22% to 19.8% w/w, mean particle size was 141.9nm, and surface charge was -30.3mV. nanoparticles loaded with drug exerted biphasic release pattern with initial burst release followed by sustained release in pH 7.4 phosphate buffer.

Xian-Jun yu*et al.*, 2009, optimized the formulation methods for loading gemcitabine into albumin nanoparticles for extended blood circulation and

improved efficacy. Two sizes of nanoparticles were prepared by desolvation method, with mean diameter of 109.7nm and 405.6nm. The drug loaded by co-precipitation and follow up adsorption method. Antitumor activity assed by using MTT assay. The two nanoparticles possessed smooth surfaces and high drug loading efficiency 11% , 13% drug loading respectively.

CHAPTER III

AIM OF WORK

CHAPTER – III

AIM OF WORK

At present cardiac diseases are the major cause of mortality, morbidity and disability. Systemic administration of drugs does not provide appropriate therapeutic drug levels in the target arteries for sufficient periods of time. Biomedical engineers have already succeeded in developing microscale instruments to open blocked arteries and to treat other cardiovascular diseases. However these tools are bulky, infection prone, and subject to other disorders. The main reason for the failure of drugs in clinical trials is the inefficacy of such an approach in providing therapeutic drug levels in the target tissues for a sustained period of time. Nanoparticles based localized drug therapy using sustained release drug delivery systems could be more effective, because it can provide higher and prolonged therapeutic drug level.

Nanotechnology explores particles between 1 and 100nm in diameter, the size of the individual particles for drug delivery of therapeutic and imaging agents may range from 2 to 1000nm. It has been confirmed that particles larger than 200nm can activate the human complement system and be cleared from the blood by kupffer cells. Additionally splenic filtration captures particles that exceed 200-250nm liver filtration captures particles greater than 150nm. For the above reasons current research ofnanopharmaceutical formulations focuses on particles less than 200nm. **(Bawarski W.E. et al., 2008)**

Cefazolin sodium is classified as a first generation cephalosporin for injectable use, it is distinguished by its effectiveness as a therapeutic agent and surgical prophylaxis. Cefazolin sodium have significant activity against gram positive and gram negative bacteria by inhibiting synthesis of bacterial cell wall in manner

similar to penicillins. It has half life of 2 hours and administered every 6 hourly during infective endocarditis and requires 1- 1.5gm dosage upto 12gm daily has been used.

The aim of the present work is to develop and characterizecefazolin sodium loaded bovine serum albumin nanoparticles with controlled particle size (< 200nm)using coacervation method. The controlled particle size is achieved by varying the process parameters like pH of the aqueous solution, polymer concentration and rpm of the magnetic stirrer. The best formulation is selected based on particle size, entrapment efficiency and invitro drug release. Best formulation is further subjected to evaluation of particle shape, stability test,infra red spectroscopy and microbiological assay.

CHAPTER IV

PLAN OF WORK

CHAPTER - IV

PLAN OF WORK

I. STANDARD CURVES FOR CEFAZOLIN

a. Preparation phosphate buffer saline pH 7.4.

b. Estimation of absorption maximum.

c. Preparation of standard curve of cefazolin.

II. DRUG –POLYMER INTERACTION STUDIES:

a. Infrared spectroscopic analysis

III. FORMULATION OF CEFAZOLIN LOADED BOVINE SERUM ALBUMIN NANOPARTICLE.

IV. CHARACTERIZATION OF CEFAZOLIN LOADED BOVINE SERUM ALBUMIN NANOPARTICLE

a. Determination of drug content.

b. Determination of drug entrapment efficiency.

c. Determination of particle size, polydispersityIndex & zeta potential.

d. Invitro release studies of cefazolin loaded bovine serum albumin nanoparticle using dialysis membrane.

e. Kinetics of drug release.

V. SELECTION AND EVALUATION OF BEST FORMULATION.

a. Infrared Spectroscopic analysis.

b. Scanning Electron Microscopy analysis.

c. Microbiological assay.

CHAPTER V

MATERIALS AND EQUIPMENTS

CHAPTER-V

MATERIALS AND EQUIPMENTS

MATERIALS USED

1. Cefazolinsodium	-	Hospira health care Private Ltd, Chennai.
2. Bovine serum albumin	-	Hi Media laboratories, Mumbai.
3. Ethanol	-	Changshuyangyuan chemicals, China.
4. Glutaraldehyde	-	Central Drug House (p) Ltd, New delhi.
5. Sodium hydroxide	-	Central Drug House (p) Ltd, New delhi.
6. Sodium chloride	-	RFCL limited, New delhi.
7. Potassium di hydrogen phosphate	-	High purity laboratory chemicals (p) Ltd,Mumbai.
8. Di sodium hydrogen phosphate	-	Nice chemicals (p) Ltd, Cochin.
9. Trypsine	-	Hi media laboratories (p) Ltd, Mumbai.

EQUIPMENTS USED

1. Electronic Weighing balance	-	A&D company, Japan.
2. UV-Visible spectrophotometer	-	Shimadzu corporation, Japan.
3. Infra red Spectroscopy	-	Shimadzu, Japan.
4. pH meter	-	M.C.Dalal, India.
5. Magnetic stirrer	-	Remi 1ML, India.
6. Lyophilizerr	-	M.C.Dalal, India.
7. Refrigerator	-	Kelvinator, India.
8. Cooling Centrifuge apparatus	-	Eppendorf centrifuge 5417R, Germany.
9. Particle size analyser	-	Nano ZS90, Malvern Instruments Ltd, UK.
10. Scanning electron microscope	-	Hitachi X650, Tokyo, Japan.
11. Ultra sonicator	-	Vibronic’s ultrasonic processor, India.

CHAPTER VI

DRUG PROFILE

CHAPTER - VI

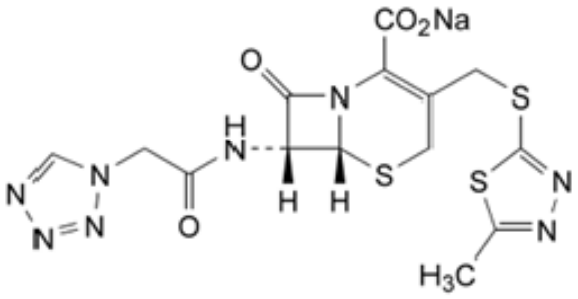
DRUG PROFILES

DRUG NAME: Cefazolin

SYNONYMS:cefazolina ,cefazolinum , sefazolin, cephazolin.

(Martindale The complete drug reference, 36th edition, page 222)

STRUCTURE:



CHEMICAL FORMULA:

$C_{14}H_{13}N_8NaO_4S_3$

IUPAC NAME:

5-thia-azabicyclo oct-2-ene-2-carboxylic acid, 3-[[(5-methyl-1, 3, 4, thiadiazol-2-yl)thio] methyl] -8- oxo -7- [[(1H-tetrazol-1-yl) acetyl] amino] - mono sodium salt.

DESCRIPTION:

Nature **:** White to off white, very hygroscopic powder.

Solubility **:** Freely soluble in water, very slightly soluble in alcohol.

Molecular weight : 476.5 gm

Melting point : 190ºc

MECHANISM OF ACTION:

Cefazolin interfere with the synthesis of bacterial cell wall. The bacteria synthesize UDP-N-acetylmuramic acid pentapeptide and UDP-N- acetylglucosamine. The peptidoglycan residues are linked together forming long strands and UDP is split off. The final step is cleavage of the terminal-alanine of the peptide chains by transpeptidases; the energy so released is utilized for establishment of linkages between peptide chains of the nieghbouring strands. This cross linking provides stability and rigidity to the cell wall. Cefazolin inhibit the transpeptidases so that cross linking does not take place. (Ancef GSK)

PHARMACOKINETICS:

Absorption:

Not appreciably absorbed from GI tract; must be administered parenterally

Volume of distribution:

Widely distributed into tissues and fluids,[a] including synovial fluid. Only low concentrations distribute into CSF. Crosses the placenta and is distributed into milk.

Metabolism:

Not metabolized

Excretion:

Excreted unchanged in urine. Approximately 60% of a dose excreted within 6 hours, and 70%-80% excreted within 24 hours in those with normal renal impairment.

Protein binding:

74 % - 86%

Half-life:

Serum half-life approximately 1.8 hours after IV administration and 2 hours after IM administration.

(www.drugs.com)

INDICATION:

Cefazolin mainly used to treat bacterial infections of the skin. It can also be used to treat bacterial infections involving the lung, bone, joint , stomach , blood, heart valve and urinary tract infection. It is clinically effective against infections caused by staphylococci and streptococci species of gram positive bacteria.May be used for surgical prophylaxis.

DOSAGE FORM :

Powder for injection IM 1gm/vial.

Powder for injection IV 1gm/vial.

DOSE :

Mild infection caused by gram positive bacteria 250-500mg every 8 hours, IV/IM.

Moderate to severe infections 500mg – 1gm every 6-8 hours, IV/IM.

Severe , life- threatening infections 1-1.5gm every 6 hours, IV/IM.

Endocarditis, 1-1.5gm every 6 hours, IV/IM.

Respiratory tract infections 500mg every 12hours, IV/IM.

Septicemia, 1-1.5gm every 6 hours, IV/IM.

ADVERSE EFFECT :

Steven – Johnson syndrome.

Hypoprothrombinaemia.

Hypersensitivity reactions.

Platelet dysfunction.

DRUG INTERACTIONS:

Aminoglycoside antibiotics – increase the risk of nephrotoxicity.

Probenecid – may increase the serum level of cefazolin.

CONTRAINDICATIONS:

Premixed injection (frozen) containing cefazolin in dextrose injection may be contraindicated in patients with known allergy to corn or corn products.

(AHFS Drug Information)

CHAPTER VII

EXCIPIENTS PROFILE

CHAPTER – VII

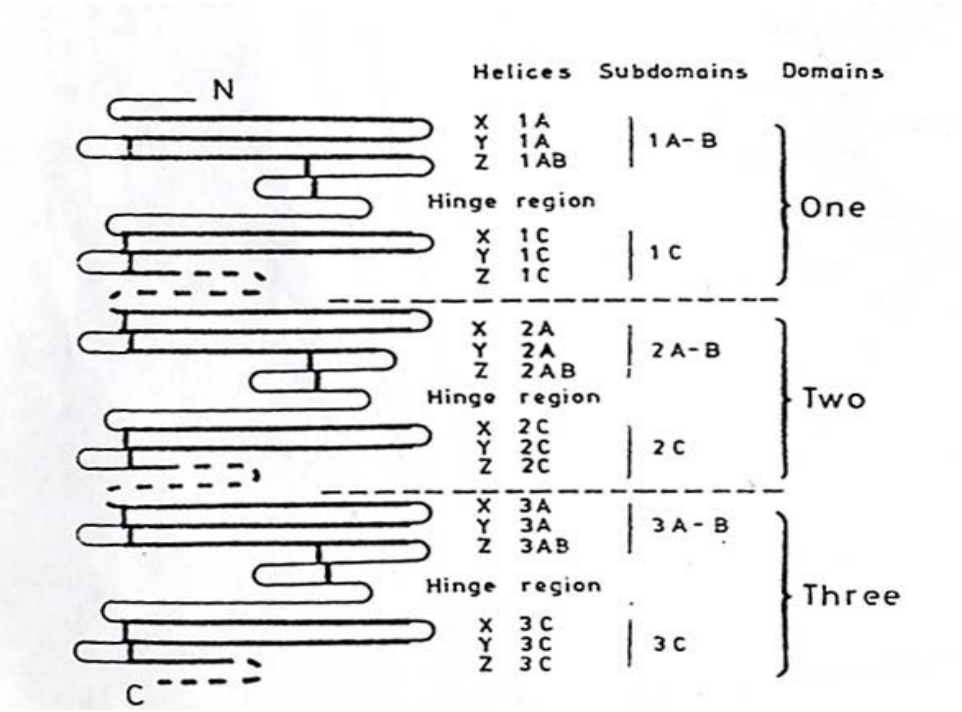
EXCIPIENTS PROFILES

ALBUMIN

Synonym : Albumin fraction V, Bovine albumin,Cohn Fraction V

Chemical name : Serum albumin

Structure : It comprises three homologous domains that assemble to form a heart shaped molecule, each domain in turn consisting of two sub domainsthat possess common structural motify(Guido barone et al., 1997).



Description : Bovine serum albumin is a single polypeptide chain consisting of about 582 amino acid residues and its sequency contains 17 disulfide bridges and 1 free cysteine in position 34(Guido barone et al., 1997).

Properties :

Appearance – White to yellow with tan to green cast powder.

Solubility - Clear to slightly hazy faint yellow to yellow green solution.

Molecular Weight : 66kDa (SambandamAnandan et al., 2013)

Functional category : stabilizing agent, therapeutic agent.

Stability and storage: albumin is a protein and is therefore susceptible to chemical degradation and denaturation by exposure to extremes of pH, high salt concentration, heat, enzymes, organic solvents and other chemical agents.

Regulatory acceptance: Included in the FDA inactive ingredients guide.

Included in parenteral products licensed in UK.

Included in the Canadian list of acceptable non-medical ingredients.

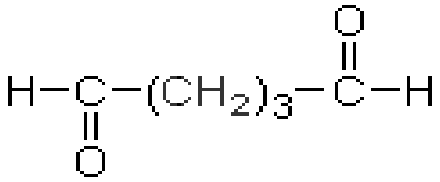
(Handbook of pharmaceutical excipients edited by Raymond C Rowe et al., sixth edition)

GLUTARALDEHYDE

Synonym : Glutaricdialdehyde, glutaral, pentane 1,5- dial , glutarol , cidax , sonacide , glutardialdehyde.

Molecular formula : $C_5H_8O_2$

Structure :



Iupac name : Pentanedial.

Description : Glutaraldehyde has fairly small molecules, each with two aldehyde groups (John A. Kiernan, 2000).

Properties :

Appearance - Colorless liquid.

Odour - pungent.

Solubility - water, benzene, ether, ethanol.

Melting point - -15ºc (25% solution).

Density - 1.06g/ml (at 20ºc).

Boiling point - 100ºc.

Storage temperature - 2º-8ºc.

(www.chemicalbook.com& www.chemicalindustry.com)

Molecular Weight : 100.1g

Use : Stabilizing agent, disinfectant, bi functional cross linking agent, electron microscopic fixative agent.

Stability and storage: Purified samples of 25% glutaraldehyde stored at -20ºc showed virtually no change in their UV absorbance characteristic even after 8 months. However solutions are very heat sensitive (sigma aldrich MSDS).

MANNITOL

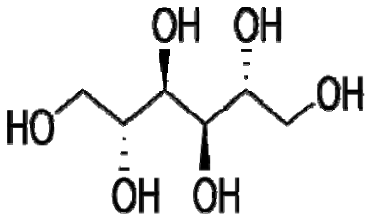
Synonyms:

- Cordycepic acid.
- Manna sugar.
- D-Mannite.
- Pearlitol.

Chemical Name:

D-Mannitol.

Chemical structure:



Empirical Formula and Molecular Weight:

$C_6H_{14}O_6$ & 182.17

Functional Category:

- Diluent.
- Sweetening agent.
- Tonicity agent.
- Cryoprotectant.

Application in Pharmaceutical Formulation:

- Mannitol is widely used in pharmaceutical formulations and food products.
- It is used as diluents (10–90% w/w) in tablet formulations.

- Mannitol is commonly used as an excipient in the manufacture of chewable tablet formulations.
- Plasticizer in soft-gelatin capsules, as a component of sustained-release tablet formulation.
- It is used as a carrier in dry powder inhalers.
- It is also used as diluents in rapidly dispersing oral dosage forms.
- It is used in food applications as a bulking agent.

Description:

- Mannitol is a white, odorless, crystalline powder, or free-flowing granules.
- It has a sweet taste.
- Microscopically, it appears as orthorhombic needles when crystallized from alcohol.
- Mannitol shows polymorphism.

Pharmacopoeial Specifications:

- Density (bulk):0.430 g/cm3.
- Density (tapped):0.734 g/cm3.
- Density (true): 1.514 g/cm3.
- Dissociation constant: pKa = 13.5 at 188C.
- Flowability: powder is cohesive, granules are free flowing.
- Melting point: 166–168^{o}C.
- Loss on drying: 40.3%.

Stability and Storage Conditions:

It should be stored in a well-closed container in a cool, dry place.

Incompatibilities:

- Mannitol solutions, 20% w/v or stronger, may be salted out by potassium chloride or sodium chloride.
- Mannitol is incompatible with xylitol infusion and may form complexes with some metals such as aluminum, copper, and iron.
- Reducing sugar impurities in mannitol have been implicated in the oxidative degradation of a peptide in a lyophilized formation.

Handling Precautions:

Mannitol may be irritant to the eyes; eye protection is recommended.

(Hand book of Pharmaceutical excipients by Raymond C Rowe -5theditions, 449-453).

CHAPTER VIII

EXPERIMENTAL PROTOCOLS

CHAPTER - VIII

EXPERIMENTAL PROTOCAL

I. STANDARD CURVEFOR CEFAZOLIN SODIUM.

a. Preparation of calibration medium (European Pharmacopoeia 2005):

Dissolve 2.38gm of disodium hydrogen phosphate, 0.91 gm of potassium di hydrogen phosphate and 8gm of sodium chloride in sufficient water to produce 1000ml.

b. Estimation of absorption maximum for cefazolin:

A known weight (100 mg) of drug (cefazolin) is dissolved in 100ml of water to make a primary stock solution (1000µg/ml). The stock solution is further diluted using a phosphate buffer salinepH (7.4)to produce 10µg/ ml concentration. The resultant solution is scanned in the range of (200-400nm) by UV Spectrophotometer(UV-1700 Shimadzu corporation, Japan) to get absorption maximum(λ max).

c. Preparation of standard curve:

The above prepared stock solution is further diluted with phosphate buffer saline pH7.4 to obtain a series of solution in theconcentration range of 5 to 25µg/ml. The absorbances of the solutions are measured at λmax 272nm by UV-spectrophotometer. A standard curves plotted using concentration on x-axis and the absorbance obtained on y-axis.

II. DRUG POLYMER INTERACTION

a. Infrared spectroscopic analysis:

Infrared spectrum analysis of drug (cefazolin), polymer and the physical mixtures of drug with polymers are obtained from FTIR spectrophotometer by KBr pellet method. The samples for FTIR analysis is prepared by grinding 99% KBr with 1% samples and then pressing the mixture into a transparent pellet.The prepared pellet is scan at the range (450– 5000cm^{-1}).The spectra obtained are compared and interpreted for the shifting of functional peaks or the appearance and disappearance of new functional peaks.(Jahanshahi M., et al., 2008).

III. FORMULATION OF CEFAZOLIN LOADED BSA NANOPARTICLES.

Cefazolin loaded nanoparticles are prepared by desolvationmethod /simple coacervation method.(LangerK. et al., 2003: WilsonB. et al., 2012)

Cefazolin (50mg), BSA (100 mg, 200mgand 300mg) are dissolved in 10ml of purified water. Then the solution pH is adjusted to pH 8-10. Add ethanol (1:2) carefully at a rate of 1ml/min under magnetic stirring. The nanoparticles are cross linked by adding 1.56µg of 25% glutaraldehyde(EspuelasMillan M.S., et al.,2004; IracheJ.M. et al., 2002) per milligram of bovine serum albumin and stirring continue for 3 hours. The nanoparticles suspension is subjected to freeze drying to obtain free flowing particles. The plain nanoparticles are prepared by the same procedure without drug.

IV. CHARACTERIZATION OF CEFAZOLIN LOADED BSA NANOPARTICLES.

The prepared Cefazolin loaded bovine serum albumin nanoparticles are evaluated in terms of drug content, entrapment efficiency, particle size and polydispersity index, zetapotential measurement, *in vitro* drug release studies and kinetics of drug release.

a. **Drug content**:

The total amount of drug content present in the formulation is determined using UV spectrophotometric analysis. Nanoparticles equivalent to 2.5mg of cefazolin sodium is taken in a 25ml standard flask. To this add 0.5ml of trypsin 1% solution, then make up to volume using phosphate buffer saline pH 7.4 and incubated for 24 hours. From this 1ml is withdrawn and suitably diluted to 10ml using buffer solution. The absorbance of the above prepared solution is examined at 272nm by UV spectrophotometer. The total drug content can be calculated from the standard curve using the formula

$$\%\text{drug content} = \frac{\text{Sample absorbance}}{\text{Standard absorbance}} \times 100$$

(Kavitadua et al., 2013)

b. Determination of Entrapment efficiency:

The amounts of cefazolin sodium encapsulated in the nanoparticles (entrapped drug) areseparated by ultracentrifugation (Eppendorf Centrifuge, 5417R, Germany) at 14,000 rpm for 90 min at 4˚C. Then, the sample is taken from the separated supernatant liquid and diluted suitably using the buffer solution and the

amount of free drug present is determined by measuring the absorbance at 272nm by UV- spectrophotometer. The amount of cefazolin sodium encapsulated in polymeric nanoparticle formulation is calculated from the difference between the total drug content and the amount of unentrapped drug remaining in the aqueous medium. The entrapment efficiency (EE) can be calculated as follows

$$\text{Entrapment efficiency} = \frac{\text{Drug content} - \text{unentrapped drug}}{\text{Drug content}} \times 100$$

(RintiBanerjee et al., 2012)

c. Determination of particle size and zetapotential:

The particle size, polydispersity index and zeta potential of drug loaded HSA nanoparticles are studied by photon correlation spectroscopy using Malvern zeta sizer 3000 (WilsonB. et al., 2012; Langer K. et al 2010). The zeta potential is measured with the same instrument by micro electrophoresisusing dip cell (Dad pariorM. et al., 2011;Langer K. et al., 2007). Prior to both measurements the samples are diluted with distilled water and measured at 25ºc with a scattering angle of 90º (Irache J.M. et al., 2001).

d. Invitro drug release studies:

The invitro drug release studies are carried out by using dialysis membrane (Li F.Q. et al., 2008; Ping yao et al., 2013). Nanoparticle equivalent to 2mg of drug is placed in a dialysis bag (cut off 5K Da). The dialysis bag is sealed at both ends then placed in 200ml ofphosphate buffer saline pH 7.4(dissolution medium). The dissolution medium is stirred continuously at 100 rpm and maintain at 37±1ºc. Withdrawn 5ml of dissolution medium at regular time

interval and same volume replaced with fresh dissolution medium. The absorbance of the samples is measured UV spectrophotometrically at a wavelength of 272nm against blank (Wilson B. et al., 2012;Fude Cui et al., 2007).

e. Kinetics of drug release:

In order to investigate the drug release mechanism from bovine serum albumin nano particles, the percentagecumulative drug release data is analyzed with following mathematical model.

- Zero-order
- First order
- Higuchi
- Hixson-Crowell cube root law
- Korsmeyer-peppas model.

• **The zero order rate Equation** describes the systems where the drug release rate is independent of itsconcentration (no equilibrium concentration are obtained).

$$\mathbf{Q_t = Q_0 + K_0 t}$$

Where,Q_t is the amount of drug dissolved in time t,Q_0 is the initial amount of drug in the solution (mosttimes, Q_0= 0) and K_0 is the zero order release constantexpressed in units of concentration/time.To study the release kinetics, data obtainedfrom *in vitro* drug release studies are plotted ascumulative amount of drug released *versus* time

•**The first order Equation** describes the release from a system where the release rateis concentration dependent.

$$\mathbf{\log C = \log C_0 – k_t / 2.303}$$

Where C is the concentration of the drug at time (t), C_0 is the initial concentration of the drugand k is the first-order release rate constant.Thedata obtained are plotted as log cumulative percentageof drug remaining *vs*. time.

• **Higuchi** describes the drug release as a diffusion process based on fick's law, square root time dependent.

$$\mathbf{Q = kt^{1/2}}$$

Where Q is the amount of drug released in time t.k is the higuchi dissolution constant. This model is based on the hypotheses that (i) initialdrug concentration in the matrix is much higher thandrug solubility; (ii) drug diffusion takes place onlyin one dimension (edge effect must be negligible);(iii) drug particles are much smaller than systemthickness; (iv) matrix swelling and dissolution arenegligible; (v) drug diffusivity is constant; and (vi)perfect sink conditions are always attained in therelease environment.The data obtained are plotted as cumulative percentagedrug release *versus* square root of time

•**Hixson and Crowell** (1931) recognized that the Particles' regular area is proportional to the cuberoot of its volume. They derived the equation

$$\mathbf{W_0^{1/3} - W_t^{1/3} = \kappa\, t}$$

Where W_0 is the initial amount of drug in the pharmaceuticaldosage form, W_t is the remaining amountof drug in the pharmaceutical dosage form at time tand κ (kappa) is a constant incorporating the surface-volumerelation.The equation describes therelease from systems where there is a change in surfacearea and diameter of particles. Tostudy the release kinetics, data obtained from *in vitro* drug release studies are plotted as cube root ofdrug percentage remaining in matrix *versus*time.

•**Korsmeyer – Peppasmodel** describes the fractionof drug release relates exponentially with respect to time.

$$M_t / M_\infty = Kt^n$$

Where M_t/M_∞ is a fraction of drug released at timet, k is the release rate constant and n is the release exponent.In this model, the value of *n* characterizes therelease mechanism of drug. To study the release kinetics,data obtained from *in vitro* drug release studies areplotted as log cumulative percentage drug release*versus*log time

Release exponent (n)	Drug transport mechanism	Rate as a function of time
0.5	Fickian diffusion	$t^{-0.5}$
0.5 < n <1	Anomalous transport	t^{n-1}
1	Case II transport	Zero order release
Higher than 1	Super case II transport	t^{n-1}

(Paulo costa & Jose manuelsousalobo, 2001).

V. SELECTION AND EVALUATION OF BEST FORMULATION.

The best formulations are selected based on the results obtained from particle size analysis, entrapment efficiency, *invitro*drug release studies.

a. Infrared spectroscopic analysis:

Infrared spectrum (IR) analysis is carried out for the selected best formulation to find out the interactions between the drug and excipients used as per the procedure mentioned on drug polymer interactions studies.

b. Morphology of BSA Nanoparticles by SEM analysis:

The morphology of the nanoparticles are observed using scanning electron microscopy. Before observation, lyophilized particles were deposited on aluminum stubs using double faced adhesive and coated with gold pallatium under an argon atmosphere using a gold sputter module in a high vacuum evaporator. (Bigucci F. et al., 2011; SatyaPrakash et al., 2010; SanghoonKo et al., 2011).

c. Microbiological assay:

The antimicrobial activity of drug and the albumin nanoparticles are analysed on the clinically isolated pathogenic strains of Staphylococcus aureus. The bacterial organisms are uniformly spread on to the sterile nutrient agar medium with sterile cotton swabs,standard and drug loaded albumin nanoparticles of 30µg/ml are poured into separate cups bored into sterile agar medium previously seeded with organisms. After allowing diffusion of solution for two hours, the plates are incubated for 12-24 hoursat 37^0C. The zone of inhibition after incubation period is observed and recorded. (Karthick Raja Namasivayam S. et al., 2013; Abdul HasanSathali A.&Sangeetha T., 2011).

CHAPTER IX

RESULTS AND DISCUSSION

CHAPTER - IX

RESULTS AND DISCUSSION

I. PREPARATION OF STANDARD CURVE FOR CEFAZOLIN.

a. Preparation of calibration medium.

The calibration medium of phosphate buffer saline pH 7.4 was prepared by using European Pharmacopeia (2005).

b. Estimation of absorption maximum.

The absorption maximum (λ max)of cefazolin was estimated by scanning the 10µg/ml concentration (using phosphate buffer saline pH 7.4) of the drug solution in UV-region (200-400nm). The obtained spectrum showed the λ max of 272nm in phosphate buffer saline pH 7.4 which was shown in **Figure. (5).**

c. Preparation of standard curve of cefazolin.

The standard curve of cefazolin was prepared using phosphate buffer saline pH 7.4.The absorbance of the solutions (5-25µg/ml) was measured in UV – spectrophotometer at 272nm. The linear correlation coefficient was obtained and it was found to be γ=0.9998. Cefazolin obeys the beer's law within the concentration range of (5-25µg/ml). The standard curve of cefazolin was shown in **Table. (1)**and**Figure. (6).**

II. DRUG –POLYMER INTERACTION STUDIES

a. Infrared spectroscopic analysis

Infra red spectroscopic analysis was performed to check out the compatibility between the drug (cefazolin) and bovine serum albumin used in the formulation of

nanoparticles. IR spectrum of the drug, polymer and the physical mixture of the drug with the polymer was shown in the **Figure. (7a-7c).** The peaks of pure drug and polymer obtained was showed in **Tables.(2a&2b)**and compared with the spectra of physical mixture, showed that there was shifting of functional peaks of BSA. The peak position of amide I band in pure BSA moved from 1652.09cm^{-1} to 1637.62cm^{-1}, amide III band moved from 1242.20cm^{-1} to 1240.27$cm^{-1.}$ The changes in these peak positions indicate that drug may interacted with BSA and causing changes in secondary structure (KavitaDua&PiyushTrivedi., 2013).

III. FORMULATION OF CEFAZOLIN LOADED BOVINE SERUM ALBUMIN NANOPARTICLES.

The desolvation of albumin with organic solvents followed by crosslinking with glutaraldehyde is a commonly used method for the preparation of protein nanoparticles (Weber C et al., 2000).

Various formulation (F1 – F18) of cefazolin were prepared using bovine serum albumin at different ratios (1:2, 1:4, 1:6) and at different pH range (pH 8, pH 9, pH 10). The formulations were prepared at two different rpm (1000, 500). The various parameters were shown in the **Table. (3).**

While addition of ethanol into the protein solution, bovine serum albumin is phase separated as its diminished water solubility. Nanoparticles were then formed from the albumin solution with the desolvation function produced by ethanol (Li FQ et al., 2008). The morphologically formed albumin nanoparticles not sufficiently stabilized could consequently redissolved again after dispersed with water and then lack an adjustment in drug release rate(Weber et al., 2000). Bovine serum albumin, including 60 amino moieties in lysine residues and 2 arginine moieties in

guanidinoside chains, might be solidified by condensation reaction with aldehyde group contained reagent. So, cross linking with glutaraldehyde was necessary for stabilization of desolvated bovine serum albumin nanoparticles. And the cross linking process plays a major role in the stability and drug release of the desolvated bovine serum albumin molecules (Irache J.M. et al., 2001).

In this work, the cross linking step was attentively performed for a period of 3 hours with 1.56µg (per mg of BSA) of glutaraldehyde. The further obtained results of drug loading and release characteristics for the biodegradable cefazolin loaded bovine serum albumin nanoparticles also elucidated the practical cross linking condition.

IV. CHARACTERIZATION OF CEFAZOLIN LOADED BOVINE SERUM ALBUMIN NANOPARTICLES.

a. Determination of drug content:

The drug content for all the formulations were found to be in the range of 91.56% to 98.18% which was shown in **Table.(4).**The results indicated that there was uniform distribution of drug in all nanoparticle formulation.

b. Determination of entrapment efficiency

The impact of drug-polymer ratio and pH on entrapment efficiency was considerably significant .The range of entrapment efficiency of formulations F1-F18 were 31.58% to 68.72% as shown in **Tables.(5a-5f)** and **Figures.(9).**

i. Effect of drug- polymer ratio

The entrapment efficiency of cefazolin loaded bovine serum albumin nanoparticles was greatly influenced by the drug-polymer ratio. The results were shown in **Tables (5a-5g)**and **Figures (9).** .

Formulations F1-F3 prepared using different ratios of bovine serum albumin (1:2 ,1:4, 1:6) at pH 9 , 1000 rpm showed the entrapment efficiency of 56.08%, 49.92%, 50.47% respectively.

The entrapment efficiency was increased in the order of F2< F3 <F1.

Formulations F4-F6 prepared using different ratios of bovine serum albumin (1:2 ,1:4, 1:6) at pH 10 , 1000 rpm showed the entrapment efficiency of 50.75%, 54.82%, 31.58% respectively.

The entrapment efficiency was increased in the order of **F6 < F4 <F5.**

Formulations F7-F9 prepared using different ratios of bovine serum albumin (1:2 ,1:4, 1:6) at pH 8 , 1000 rpm showed the entrapment efficiency of 67.81%, 65.30%, 58.30% respectively.

The entrapment efficiency was increased in the order of **F9< F8 <F7**.

Formulations F10-F12 prepared using different ratios of bovine serum albumin (1:2 ,1:4, 1:6) at pH 9 , 500 rpm showed the entrapment efficiency of 60.74%, 36.61%, 40.68% respectively.

The entrapment efficiency was increased in the order of **F11 < F12 <F10**.

Formulations F13-F15 prepared using different ratios of bovine serum albumin (1:2 ,1:4, 1:6) at pH 10 , 500 rpm showed the entrapment efficiency of 42.53%,49.52 %,49.38 % respectively.

The entrapment efficiency was increased in the order of **F13 < F15 <F14.**

Formulations F16-F18 prepared using different ratios of bovine serum albumin (1:2 ,1:4, 1:6) at pH 8, 500 rpm showed the entrapment efficiency of 47.06%,50.98 %,68.72 % respectively.

The entrapment efficiency was increased in the order of **F16 < F17 <F18**.

From the above results it was observed nanoparticle formulation **F7, F8, F17** has high entrapment efficiency of **67.81%, 65.30%, 68.72%** respectively than other formulations.

The higher entrapment of the drug would be mainly attributed to the desolvated phase transition and desolvation technique of nanoencapsulation. As for the matrix drug delivery system, hydrophobic interactions can affect the drug loading properties. The hydrophobic tendency of protein particles induced by glutaraldehyde might also hinder the encapsulation of the hydrophilic water soluble drug (Li F.Q. et al., 2008).

ii. Effect of pH

Bovine serum albumin is a protein with amino group and acid group has isoelectric pH of 4.5. Protein was relatively stables in the medium with pH around its isoelectric point. The entrapment efficiency of bovine serum albumin nanoparticles cross linked with glutaraldehyde might be affected by the pH value of the medium (LiF.Q. et al., 2008).

Formulations F1-F3 prepared at pH 9 using different ratios of bovine serum albumin (1:2 ,1:4, 1:6), 1000 rpm showed the entrapment efficiency of 56.08%, 49.92%, 50.47% respectively.

The entrapment efficiency was increased in the order of **F2 < F3 <F1.**

Formulations F4-F6 prepared at pH 10 using different ratios of bovine serum albumin (1:2 ,1:4, 1:6), 1000 rpm showed the entrapment efficiency of 50.75%, 54.82%, 31.58% respectively.

The entrapment efficiency was increased in the order of**F6 < F4 <F5.**

Formulations F7-F9 prepared at pH 8 using different ratios of bovine serum albumin (1:2 ,1:4, 1:6), 1000 rpm showed the entrapment efficiency of 67.81%, 65.30%, 58.30% respectively.

The entrapment efficiency was increased in the order of **F9< F8 <F7**.

Formulations F10-F12 prepared at pH 9 using different ratios of bovine serum albumin (1:2 ,1:4, 1:6), 500 rpm showed the entrapment efficiency of 60.74%, 36.61%, 40.68% respectively.

The entrapment efficiency was increased in the order of **F11 < F12 <F10.**

Formulations F13-F15 prepared at pH 10 using different ratios of bovine serum albumin (1:2 ,1:4, 1:6), 500 rpm showed the entrapment efficiency of 42.53%,49.52 %,49.38 % respectively.

The entrapment efficiency was increased in the order of **F13 < F15 <F14**.

Formulations F16-F18 prepared at pH 8 using different ratios of bovine serum albumin (1:2 ,1:4, 1:6), 500 rpm showed the entrapment efficiency of 47.06%,50.98 %,68.72 % respectively.

The entrapment efficiency was increased in the order of **F16 < F17 <F18.**

From the above results the higher entrapment efficiency was observed at pH value 8, further increased in pH values decreased the entrapment efficiency. The

decreased entrapment efficiency might be concerned with the accelerated cross linking step in alkaline condition (Schiff base formed) (Li FQ et al., 2008).

c. Determination of particle size and zeta potential

The particle size,polydispersity index and zeta potential of drug (cefazolin) loaded bovine serum albumin nanoparticles were studied by photon correlation spectroscopy(Wilson B et al., 2012; Langer K et al., 2010). The mean particle diameter of all formulation from F1-F18 was found to be in the range of 46.9nm-345.3nm.

The mean particle diameter of cefazolin loaded bovine serum albumin nanoparticle was influenced by the drug- polymer ratio, pH and rotation per minutes of thestirrer, the results were shown in **Tables.(6a-6f)&Figures.(10a-10j).**

i. Effect of drug- polymer ratio

The influence of different ratio of polymer on cefazolin loaded bovine serum albumin nanoparticles was investigated; the results were shown in **Tables.(6a-6f)&Figures.(10a-10j).**

Formulations F1-F3 prepared using different ratio of bovine serum albumin (1:2 , 1:4 ,1:6)at pH 9, 1000 rpm showed the particle size of 136.9nm , 232.4nm, 265.9nm respectively.

The mean particle diameter was increased in the order of **F1<F2<F3**.

Formulations F4-F6 prepared using different ratio of bovine serum albumin (1:2 , 1:4 ,1:6)at pH 10, 1000 rpm showed the particle size of 192.7nm , 220.1nm, 240.7nm respectively.

The mean particle diameter was increased in the order of **F4<F5<F6**.

Formulations F7-F9 prepared using different ratio of bovine serum albumin (1:2 , 1:4 ,1:6)at pH 8, 1000 rpm showed the particle size of 46.9nm , 252.0nm, 288.2nm respectively.

The mean particle diameter was increased in the order of **F7<F8<F9**.

Formulations F10-F12 prepared using different ratio of bovine serum albumin (1:2 , 1:4 ,1:6)at pH 9, 500 rpm showed the particle size of 142.2nm , 316.2nm, 334.2nm respectively.

The mean particle diameter was increased in the order of **F10<F11<F12**.

Formulations F13-F15 prepared using different ratio of bovine serum albumin (1:2 , 1:4 ,1:6)at pH 10, 500 rpm showed the particle size of 209nm , 305.9nm, 309.7nm respectively.

The mean particle diameter was increased in the order of **F13<F14<F15**.

Formulations F16-F18 prepared using different ratio of bovine serum albumin (1:2 , 1:4 ,1:6)at pH 8, 500 rpm showed the particle size of 49.03nm , 138.3nm, 345.3nm respectively.

The mean particle diameter was increased in the order of **F16<F17<F18**.

The particle size of all the formulation increased in the order of

1:2<1:4<1:6

It was concluded that an increase in polymer concentration increased the particle size of cefazolin loaded bovine serum albumin nanoparticles. Because

increases in bovine serum albumin increase the chances of coagulation, especially, the protein molecule have had more chances to undergo electrostatic and hydrophobic interactions. Larger hydrophobic interaction of bovine serum albumin increased the coagulation of the molecules and subsequently related in larger particles.(sanghoonko et al., 2011).

ii. Effect of pH on particle size

The mean particle diameter of bovine serum albumin nanoparticles loaded with cefazolin were prepared at three different pH conditions (8,9,10) , using different concentration of polymer(1%,2%,3%) at two different rpm(1000,500). The results were shown in **Tables.(6a-6f)&Figures.(10a-10j).**

Formulations F1-F3 prepared at pH 9 using different ratio of bovine serum albumin (1:2 , 1:4 ,1:6), 1000 rpm showed the particle size of 136.9nm , 232.4nm, 265.9nm respectively.

The mean particle diameter was increased in the order of **F1<F2<F3**.

Formulations F4-F6 prepared at pH 10 using different ratio of bovine serum albumin (1:2 , 1:4 ,1:6), 1000 rpm showed the particle size of 192.7nm , 220.1nm, 240.7nm respectively.

The mean particle diameter was increased in the order of **F4<F5<F6**.

Formulations F7-F9 prepared at pH 8 using different ratio of bovine serum albumin (1:2 , 1:4 ,1:6), 1000 rpm showed the particle size of 46.9nm , 252.0nm, 288.2nm respectively.

The mean particle diameter was increased in the order of **F7<F8<F9**.

Formulations F10-F12 prepared at pH 9 using different ratio of bovine serum albumin (1:2 , 1:4 ,1:6), 500 rpm showed the particle size of 142.2nm , 316.2nm, 334.2nm respectively.

The mean particle diameter was increased in the order of **F10<F11<F12**.

Formulations F13-F15 prepared at pH 10 using different ratio of bovine serum albumin (1:2 , 1:4 ,1:6), 500 rpm showed the particle size of 209nm , 305.9nm, 309.7nm respectively.

The mean particle diameter was increased in the order of **F13<F14<F15**.

Formulations F16-F18 prepared at pH 8 using different ratio of bovine serum albumin (1:2 , 1:4 ,1:6), 500 rpm showed the particle size of 49.03nm , 138.3nm, 345.3nm respectively.

The mean particle diameter was increased in the order of **F16<F17<F18**.

From the above results it was observed that increase in pH decrease in particle size (Langer K et al.,2003). The control of the BSA particle size can be explained by their surface charge and surface hydrophobicity. Surface hydrophobicity dictates the propensity to bind non-polar amino acid groups to a hydrophobic part of its surface. Hydrophobic interactions lead to a coagulations among protein molecules, resulting in size increase. Under basic conditions such as pH 10 electrostatic interactions increase but hydrophobic interaction decrease. In addition at basic pH hydrogen bonding increase results in a decrease in the hydrophobicity. At pH 10 there was a decrease in the hydrophobic interactions. This results support our hypothesis with regards to controlling hydrophobic interaction as a means for controlling the size of bovine serum albumin nanoparticles(SanghoonKoet al., 2011).

iii. Effect of rpm

The mean particle diameter of bovine serum albumin nanoparticles loaded with cefazolin were prepared at two different rpm(1000,500), using different concentration of polymer(1%,2%,3%) at three different pH conditions (8,9,10). The results were shown in **Tables. (6a-6f)&Figures.(10a-10j).**

Formulations F1-F3 prepared at 1000 rpm using different ratio of bovine serum albumin (1:2, 1:4, 1:6) inthe pH 9 showed the particle size of 136.9nm, 232.4nm, 265.9nm respectively.

The mean particle diameter was increased in the order of **F1<F2<F3**.

Formulations F4-F6 prepared at 1000 rpm using different ratio of bovine serum albumin (1:2, 1:4, 1:6) in thepH 10 showed the particle size of 192.7nm, 220.1nm, 240.7nm respectively.

The mean particle diameter was increased in the order of **F4<F5<F6**.

Formulations F7-F9 prepared at1000 rpm using different ratio of bovine serum albumin (1:2, 1:4, 1:6) in the pH 8 showed the particle size of 46.9nm, 252.0nm, 288.2nm respectively.

The mean particle diameter was increased in the order of **F7<F8<F9**.

Formulations F10-F12 prepared at 500 rpm using different ratio of bovine serum albumin (1:2, 1:4, 1:6) in thepH 9 showed the particle size of 142.2nm , 316.2nm, 334.2nm respectively.

The mean particle diameter was increased in the order of **F10<F11<F12.**

Formulations F13-F15 prepared at 500 rpm using different ratio of bovine serum albumin (1:2, 1:4, 1:6) in thepH 10 showed the particle size of 209nm , 305.9nm, 309.7nm respectively.

The mean particle diameter was increased in the order of **F13<F14<F15**.

Formulations F16-F18 prepared at 500 rpm using different ratio of bovine serum albumin (1:2, 1:4, 1:6) in thepH 8 showed the particle size of 49.03nm, 138.3nm, 345.3nm respectively.

The mean particle diameter was increased in the order of **F16<F17<F18**.

Formulations (**F1-F9)** were prepared at 1000rpm showed the mean particle diameter in the ranges of **46.9nm to 288.2nm**.

Further formulations (**F10-F18)** were prepared at 500rpm showed the mean particle diameter in the ranges of **49.03nm to 345.3nm**.

From the above results it was observed that the formulations F1(pH 9, 1:2, 1000rpm), F7 (pH8, 1:2, 1000rpm), F10(pH9, 1:2, 500rpm), F16 (pH8, 1:2, 500rpm), F17 (pH8, 1:4, 500rpm) showed the mean particle diameter of 136.9nm, 46.9nm, 142.2nm, 49.03nm, 138.3nm respectively.

The mean particle diameter of the formulations (F1-F18)were decreased with increased rpm (Jahanshahi M et al., 2006). Because at higher rpm distribution of desolvating agent (ethanol) occurs uniformly which inhibit aggregate formation (Kavitadua, Piyushtrivedi 2013).

Polydispersity index

The most important characterization parameter, also called Polydispersity Index (PI)which governs the physical stability of nanoparticles and should be as low as possible for the long term stability of nanoparticles.

a) PI value of 0.1–0.25 indicates a fairly narrow size distribution

b) PI value greater than 0.5 indicates a very broad distribution

Polydispersity index values for all the formulations were shown in **Tables. (6a-6f)** and **Figure.(12a,12b).**

The polydispersity index of formulations (F1-F9) prepared using different ratio of polymer (1:2, 1:4, 1:6) at 1000 rpm in different pH (8, 9,10) was found to be in the range of 0.183-0.408.

The polydispersity index of formulations (F10-F18) prepared using different ratio of polymer (1:2, 1:4, 1:6) at 500 rpm in different pH (8, 9,10) was found to be in the range of 0.164-0.608.

It was observed all the formulations haspolydispersity index smaller than 0.5 which indicated anarrow size distribution except F16 (0.608).

Zeta potential

The stability of a prepared bovine serum albumin nanoparticle formulations were measured in terms of zeta potentials. It reflects the electrical potential of particles and is influenced by the composition of the particle and the medium in which it is dispersed (Jahanshahi M et al., 2008).Greater the surface charge, more repulsion between particles, which in turn produce a stables suspension by reducing the neutral tendency to aggregate (Wilson B et al., 2012).

The zeta potential of formulations (F1-F19) prepared using different ratio of polymer (1:2, 1:4, 1:6) at 1000 rpm in different pH (8, 9,10) was found to be in the range of (-6.78mV to 2.51mV).

The zeta potential of formulations (F1-F19) prepared using different ratio of polymer (1:2, 1:4, 1:6) at 500 rpm in different pH (8, 9,10) was found to be in the range of (-6.36mV to -16.5mV).

The results were shown in **Tables.(6g, 6h) &Figures. (11a-11i & 12a,12b).** Nanoparticles with a zeta potential above ±30 mV have been shown to be stables in suspension, as the surface charge prevents aggregation of the particles (Jahanshahi M et al., 2008).

Zeta potential is significantly affected by the amount of protein and cefazolin. In the coacervation process,glutaraldehyde and cefazolin (highly protein bound) both compete for the amino groups of protein. We fixed the concentration of glutaraldehyde at 1.56µg/mg of bovine serum albumin. At this concentration sufficient number of amino groups is free for cefazolin binding even after binding of all the glutaraldehyde to albumin (Rintibanerjee et al., 2005).

d. Invitro release studies

The invitro release of cefazolin from the albumin nanoparticles was studied by the dialysis bag diffusion technique. The release study was performed at phosphate buffer saline pH 7.4 for 12 hours. The dialysis bag retained nanoparticles and allow the diffusion of the drug immediately into the receptor compartment. The results were shown in **Tables.(7a-7f)** and **Figures.(13a-13f).**

The formulations F1-F3 prepared using different ratio (1:2, 1:4, 1:6) of bovine serum albumin in pH 9 at 1000rpm exhibited an initial burst release of 14.72%,

13.33%, 14.11% at 0.5 hour and 62.41%, 64.05%, 62.52% at 12 hours respectively. The cumulative percentage of drug release was decreased in the order of **F3>F1>F2.**

The formulations F4-F6 prepared using different ratio (1:2, 1:4, 1:6) of bovine serum albumin in pH 10 at 1000rpm exhibited an initial burst release of 13.19%, 11.51%, 15.45% at 0.5 hour and 63.90%, 59.34%, 72.24% at 12 hours respectively. The cumulative percentage of drug release was decreased in the order of **F6>F4>F5**.

The formulations F7-F9 prepared using different ratio (1:2, 1:4, 1:6) of bovine serum albumin in pH 8 at 1000rpm exhibited an initial burst release of 6.35%, 14.30%, 16.68% at 0.5 hour and 46.69%, 56.37%, 61.39% at 12 hours respectively. The cumulative percentage of drug release was decreased in the order of **F9>F8>F7**.

The formulations F10-F12 prepared using different ratio (1:2, 1:4, 1:6) of bovine serum albumin in pH 9 at 500rpm exhibited an initial burst release of 11.93%, 19.89%, 15.42% at 0.5 hour and 55.10%, 76.52%, 64.26% at 12 hours respectively. The cumulative percentage of drug release was decreased in the order of **F11>F12>F10**.

The formulations F13-F15 prepared using different ratio (1:2, 1:4, 1:6) of bovine serum albumin in pH 10 at 500rpm exhibited an initial burst release of 23.10%, 21.28%, 24.22% at 0.5 hour and 78.02%, 70.40%, 70.58% at 12 hours respectively. The cumulative percentage of drug release was decreased in the order of **F13> F15 > F14**.

The formulations F16-F18 prepared using different ratio (1:2, 1:4, 1:6) of bovine serum albumin in pH 8 at 500rpm exhibited an initial burst release of 19.75%, 13.89%, 16.26% at 0.5 hour and 62.17%, 47.78%, 57.36% at 12 hours respectively.

The cumulative percentage of drug release was decreased in the order of **F16 > F18 > F17**.

From the above results it was observed that all the formulations showed biphasic release. All the formulations showed about 20% of drug release quickly from cefazolin loaded bovine serum albumin nanoparticles with the first 0.5 hour and then followed by relatively slow release rate. This initial burst release was caused by the drug dispersed close to the nanoparticle surface. This part of adsorbed drug could be easily desorbed from the outer layer and diffused out (Li FQ et al., 2008). The drug incorporated into the inner core of the protein matrix contributed for slow release phase (Wilson B et al., 2012) & (Irache J.M et al., 2001). The albumin matrix of the nanoparticle is stables during invitro drug release, it was reasonable owing to the insolubility of denatured bovine serum albumin (Fude cui et al., 2007)

e. Kinetics of drug release

The results obtained from the *in vitro* release studies were attempted to fit into various mathematical models as follows:

a) Zero order rate kinetics - Cumulative percentage drug release Vs time

b) First order rate kinetics- Log cumulative percentage drug remaining Vs time

c) Higuchi classical diffusion model- Cumulative percentage drug release Vs square root of time

d) Hixon Crowell erosion equation- Cube root of percentage drug remaining Vs time

e) KorsmeyerPeppas exponential equation- Log cumulative percentage drug release Vs log time

Various plots of zero order, first order, Higuchi matrix, Korsmeyer - Peppas and Hixon – Crowell were shown in **Figures.(18a-18f)**. The regression coefficient (r^2) and n values were shown in**Tables.(8a,8b).**

Higuchi describes drug release as a diffusion process based on Fick's law, which is square root of time dependent. The amount of drug release from cefazolin loaded bovine serum albumin nanoparticle formulations (F1-F18) showed a linear relationship with square root of time. Hence, the drug release rate could be expressed by Higuchi diffusion model (r^2=0.896 to 0.973). The high correlation coefficients were obtained for the first order drug release kinetics for all the formulations ranging from (r^2 = 0.804to 0.925).

Different values of n for cylindrical, spherical and slab of geometrics are available in the literature (Costa P, Sousa lobo JM 2001). For spheres, values of 0.5, 0.5 < n < 1.0, 1.0 and higher than 1.0 are related to Fickian diffusion, anomalous, case II transport and super case II transport respectively. According to the data presented in the **Tables. (8a, 8b)** the values of exponent n were within 0.5 which indicated that the drug release mechanism followed pure Fickian diffusion. This report was in accordance with the earlier studies of Wilson B et al., 2012.

V. SELECTION AND EVALUATION OF BEST FORMULATION.

From the above results F7 was selected as the best formulation showing

Particle size	:	46.9nm
Entrapment efficiency:		67.81%
Invitro drug release	:	46.69% at 12 hours
Release kinetics	:	closest to higuchi model

a. Infra red spectrum analysis

IR spectrum of the best formulations (F7) was recorded and shown in the **Figure.(7d)**. The IR spectrum of pure BSA obtained was compared with the spectra of best formulations, showed that there was shifting ofsome functional peak. The peak position of amino group in BSA moved from 3418.94cm^{-1} to 3277.27cm^{-1} after formulation of nanoparticles.This result indicatesthe shifting was due to crosslinking of amino groups by glutaraldehyde (KavitaDua&PiyushTrivedi., 2013).

b. Morphology of polymeric nanoparticles by SEM analysis

In order to characterize the morphology of nanoparticle formulations SEM analysis was performed. The SEM Photograph of the selected best formulations F7 (cefazolin + 1% BSA + pH 8) were shown in **Figure.(19).**The results indicated that the nanoparticles are spherical in shape and below 0.5µm in size

c. Microbiological assay

The zone of inhibition measured was compared with that of standard and the study indicated that cefazolin retained its antimicrobial efficacy even after formulated as nanoparticles **Figure.(20)**(Abdul HasanSathali A.&Sangeetha T., 2011)**.** The antibacterial activity against staphylococcus aureus revealed there was a sharp increase in zone of inhibition at increased time period and enhanced zone of inhibition was recorded after 24 hours with 36mm.(Karthick Raja Namasivayam S. et al., 2013).

TABLES & FIGURES

TABLE 1.CALIBRATION OF CEFAZOLIN SODIUM USING PHOSPHATE BUFFER SALINE pH 7.4

CONCENTRATION µg/ml	ABSORBANCE ± SD
5	0.132 ± 0.010
10	0.257 ± 0.003
15	0.378 ± 0.006
20	0.496 ± 0.014
25	0.610 ± 0.012

n = 3* γ = 0.9998 ± 0.0003

TABLE 2(a). FT-IR PEAKS OF CEFAZOLIN.

S.NO	CHARACTERISTICS	WAVENUMBER
1	NH stretching	Near 3422.8 cm^{-1} and 3295.69 cm^{-1}
2	C=O (carboxylate) stretching	1761.07 cm^{-1}
3	C=O (amide) stretching	1668.48 cm^{-1}
4	COO^- stretching	1599.04 cm^{-1} and 1388.79 cm^{-1}
5	C-S stretching	2934.79 cm^{-1}
6	C-N stretching	1238.41 cm^{-1}, 1183.37 cm^{-1}, 1104.28 cm^{-1}, 1075.35 cm^{-1}
7	secondary amide	1541.18 cm^{-1}
8	CH aliphatic stretching	2934.79 cm^{-1}
9	C=C aromatic stretching	1599.04 cm^{-1}, 1541.18 cm^{-1}, 1491.99 cm^{-1}

TABLE 2(b). FT-IR PEAKS OF BOVINE SERUM ALBUMIN.

S.NO	CHARACTERISTICS	WAVENUMBER
1	N-H bending	Near 3418.93 cm^{-1}
2	C=O stretching (amide-I)	1652.09 cm^{-1}
3	N-H bending C-N stretching (amide-II)	1541.18 cm^{-1}
4	N-H bending C-N stretching (amide-III)	1242.20 cm^{-1}
5	COO^-	1396.58 cm^{-1}

TABLE 3.VARIUOS PARAMETERS OF CEFAZOLIN LOADED BOVINE SERUM ALBUMIN NANO PARTICLE.

FORMULATION CODE	DRUG POLYMER RATIO	DRUG (mg)	POLYMER (mg)	RPM	pH
F1	1: 2	50	100	1000	9
F2	1: 4	50	200	1000	9
F3	1: 6	50	300	1000	9
F4	1: 2	50	100	1000	10
F5	1: 4	50	200	1000	10
F6	1: 6	50	300	1000	10
F7	1: 2	50	100	1000	8
F8	1: 4	50	200	1000	8
F9	1: 6	50	300	1000	8
F10	1: 2	50	100	500	9
F11	1: 4	50	200	500	9
F12	1: 6	50	300	500	9
F13	1: 2	50	100	500	10
F14	1: 4	50	200	500	10
F15	1: 6	50	300	500	10
F16	1: 2	50	100	500	8
F17	1: 4	50	200	500	8
F18	1: 6	50	300	500	8

TABLE 4.DRUG CONTENT OF CEFAZOLIN LOADED BOVINE SERUM ALBUMIN NANOPARTICLES.

S.NO	FORMULATION CODE	DRUG CONTENT(%) ± SD
1	F1	92.99 ± 0.840
2	F2	96.10 ± 1.375
3	F3	95.08 ± 0.330
4	F4	93.78 ± 0.478
5	F5	91.56 ± 0.399
6	F6	90.78 ± 0.733
7	F7	94.68 ± 0.183
8	F8	96.10 ± 0.420
9	F9	94.16 ± 0.550
10	F10	97.80 ± 0.243
11	F11	96.36 ± 0.242
12	F12	95.32 ± 0.275
13	F13	96.78 ± 1.606
14	F14	95.32 ± 0.159
15	F15	98.18 ± 0.875
16	F16	97.92 ± 0.459
17	F17	96.10 ± 0.840
18	F18	97.28 ± 0.159

$n = 3^{*}$

TABLE 5a. ENTRAPMENT EFFICIENCY OF CEFAZOLIN SODIUM LOADED BSA NANO PARTICLES FORMULATED AT pH 9, 1000RPM

S.NO	FORMULATION CODE	DRUG : POLYMER RATIO	ENTRAPMENT EFFICIENCY(%) ± SD
1	F1	1:2	56.08 ± 0.762
2	F2	1:4	49.92 ± 0.622
3	F3	1:6	50.47 ± 0.344

n = 3*

TABLE 5b. ENTRAPMENT EFFICIENCY OF CEFAZOLIN SODIUM LOADED BSA NANO PARTICLES FORMULATED AT pH 10, 1000RPM

S.NO	FORMULATION CODE	DRUG : POLYMER RATIO	ENTRAPMENT EFFICIENCY(%) ± SD
1	F4	1:2	50.75 ± 0.266
2	F5	1:4	54.82 ± 0.461
3	F6	1:6	31.58 ± 0.805

n = 3*

TABLE 5c. ENTRAPMENT EFFICIENCY OF CEFAZOLIN SODIUM LOADED BSA NANO PARTICLES FORMULATED AT pH 8, 1000RPM

S.NO	FORMULATION CODE	DRUG : POLYMER RATIO	ENTRAPMENT EFFICIENCY(%) ± SD
1	F7	1:2	67.81 ± 0.849
2	F8	1:4	65.30 ± 1.453
3	F9	1:6	58.30 ± 1.063

n = 3*

TABLE 5d. ENTRAPMENT EFFICIENCY OF CEFAZOLIN SODIUM LOADED BSA NANO PARTICLES FORMULATED AT pH 9, 500RPM

S.NO	FORMULATION CODE	DRUG : POLYMER RATIO	ENTRAPMENT EFFICIENCY(%) ± SD
1	F10	1:2	60.74 ± 0.477
2	F11	1:4	36.61 ± 1.752
3	F12	1:6	40.68 ± 1.707

n = 3*

TABLE 5e. ENTRAPMENT EFFICIENCY OF CEFAZOLIN SODIUM LOADED BSA NANO PARTICLES FORMULATED AT pH 10, 500RPM

S.NO	FORMULATION CODE	DRUG : POLYMER RATIO	ENTRAPMENT EFFICIENCY(%) ± SD
1	F13	1:2	42.53 ± 1.377
2	F14	1:4	49.52 ± 0.915
3	F15	1:6	49.38 ± 1.537

n = 3*

TABLE 5f. ENTRAPMENT EFFICIENCY OF CEFAZOLIN SODIUM LOADED BSA NANO PARTICLES FORMULATED AT pH 8, 500RPM

S.NO	FORMULATION CODE	DRUG : POLYMER RATIO	ENTRAPMENT EFFICIENCY(%) ± SD
1	F16	1:2	50.98 ± 0.737
2	F17	1:4	68.72 ± 1.870
3	F18	1:6	47.06 ± 1.557

n = 3*

TABLE 6a. PARTICLE SIZE AND PDI OF CEFAZOLIN LOADED BOVINE SERUM ALBUMIN NANOPARTICLES FORMULATED AT pH 9,1000rpm.

FORMULATION CODE	DRUG : POLYMER RATIO	MEAN DIAMETER (nm)	PDI
F 1	1:2	136.9	0.245
F 2	1:4	288	0.214
F 3	1:6	390.6	0.408

TABLE 6b. PARTICLE SIZE AND PDI OF CEFAZOLIN LOADED BOVINE SERUM ALBUMIN NANOPARTICLES FORMULATED AT pH 10,1000rpm.

FORMULATION CODE	DRUG : POLYMER RATIO	MEAN DIAMETER (nm)	PDI
F 4	1:2	309.7	0.302
F 5	1:4	316.2	0.183
F 6	1:6	522.9	0.281

TABLE 6c. PARTICLE SIZE AND PDI OF CEFAZOLIN LOADED BOVINE SERUM ALBUMIN NANOPARTICLES FORMULATED AT pH 8,1000rpm.

FORMULATION CODE	DRUG : POLYMER RATIO	MEAN DIAMETER (nm)	PDI
F 7	1:2	46.9	0.265
F 8	1:4	232.4	0.239
F 9	1:6	305.9	0.255

TABLE 6d. PARTICLE SIZE AND PDI OF CEFAZOLIN LOADED BOVINE SERUM ALBUMIN NANOPARTICLES FORMULATED AT pH 9,500rpm.

FORMULATION CODE	DRUG : POLYMER RATIO	MEAN DIAMETER (nm)	PDI
F 10	1:2	142.2	0.309
F 11	1:4	345.3	0.213
F 12	1:6	497.6	0.502

TABLE 6e. PARTICLE SIZE AND PDI OF CEFAZOLIN LOADED BOVINE SERUM ALBUMIN NANOPARTICLES FORMULATED AT pH 10,500rpm.

FORMULATION CODE	DRUG : POLYMER RATIO	MEAN DIAMETER (nm)	PDI
F 13	1:2	334.2	0.482
F 14	1:4	485.5	0.164
F 15	1:6	552.2	0.464

TABLE 6f. PARTICLE SIZE AND PDI OF CEFAZOLIN LOADED BOVINE SERUM ALBUMIN NANOPARTICLES FORMULATED AT pH 8, 500rpm.

FORMULATION CODE	DRUG : POLYMER RATIO	MEAN DIAMETER (nm)	PDI
F 16	1:2	49.03	0.608
F 17	1:4	138.3	0.327
F 18	1:6	363.5	0.379

TABLE 6g. ZETA POTENTIAL OF CEFAZOLIN LOADED BOVINE SERUM ALBUMIN NANOPARTICLES FORMULATED AT 1000rpm.

FORMULATION CODE	pH	DRUG : POLYMER RATIO	ZETA POTENTIAL (mV)
F 1	9	1:2	-6.78
F 2		1:4	-18.2
F 3		1:6	2.51
F 4	10	1:2	-7.99
F 5		1:4	-9.30
F 6		1:6	-13.4
F 7	8	1:2	-10.4
F 8		1:4	-12.4
F 9		1:6	-14.9

TABLE 6h. ZETA POTENTIAL OF CEFAZOLIN LOADED BOVINE SERUM ALBUMIN NANOPARTICLES FORMULATED AT 500rpm.

FORMULATION CODE	pH	DRUG : POLYMER RATIO	ZETA POTENTIAL (mV)
F 10	9	1:2	-8.96
F 11		1:4	-11.7
F 12		1:6	-7.5
F 13	10	1:2	-13.7
F 14		1:4	-6.36
F 15		1:6	-10.2
F 16	8	1:2	-15
F 17		1:4	-13.3
F 18		1:6	-16.5

TABLE 7a. COMPARATIVE *INVITRO* DRUG RELEASE STUDY OF CEFAZOLIN LOADED BOVINE SERUM ALBUMIN NANOPARTICLE FORMULATIONS F1, F2, F3.

TIME IN HOURS	CUMULATIVE % DRUG RELEASE ± SD		
	F1	F2	F3
0.5	14.72 ± 0.395	13.33 ±0.395	14.11 ± 0.522
1	27.65 ± 1.304	29.15 ± 0.202	27.08 ± 0.516
1.5	32.94 ±1.086	34.20 ± 0.188	33.19 ± 1.208
2	37.93 ± 0.582	39.08 ± 0.202	38.18 ± 0.432
3	42.18 ± 0.791	43.49 ± 0.209	42.02 ± 0.551
4	46.23 ± 1.001	47.17 ± 0.359	45.52 ± 0.564
5	49.39 ± 0.436	50.06 ± 0.519	48.38 ± 0.477
6	50.92 ± 1.031	52.03 ± 0.532	50.72 ± 0.660
7	53.72 ± 0.801	54.29 ± 0.701	52.96 ± 0.831
8	55.87 ± 0.671	56.59 ± 0.891	54.81 ± 0.680
9	58.04 ± 0.654	58.77 ± 0.740	56.82 ± 0.620
10	59.67 ± 0.333	60.84 ± 0.757	58.70 ± 0.567
11	61.04 ± 0.526	62.51 ± 0.374	60.60 ± 0.580
12	62.41 ± 0.420	64.05 ± 0.237	62.52 ± 0.655

n = 3*

TABLE 7b. COMPARATIVE *INVITRO* DRUG RELEASE STUDY OF CEFAZOLIN LOADED BOVINE SERUM ALBUMIN NANOPARTICLE FORMULATIONS F4, F5, F6.

TIME IN HOURS	CUMULATIVE % DRUG RELEASE ± SD		
	F4	F5	F6
0.5	13.19 ±0.342	11.51 ± 0.684	15.42 ± 1.294
1	27.20 ±0.525	23.11 ± 0.577	32.70 ± 0.551
1.5	34.71 ± 0.701	30.80 ± 1.184	40.90 ± 0.701
2	39.74 ± 0.620	36.29 ± 1.454	47.06 ± 0.356
3	43.89 ± 0.556	40.93 ± 1.821	51.67 ± 0.701
4	47.29 ± 0.449	44.96 ± 1.155	54.69 ± 1.237
5	49.78 ± 0.578	47.95 ± 0.921	56.64 ± 1.561
6	52.16 ± 0.591	50.71 ± 0.800	59.17 ± 1.598
7	54.56 ± 0.604	52.82 ± 0.318	61.73 ± 1.374
8	56.73 ± 0.431	54.25 ± 0.165	63.90 ± 0.845
9	58.77 ± 0.439	55.55 ± 0.044	66.24 ± 0.531
10	60.56 ± 0.511	56.99 ± 0.166	68.45 ± 0.551
11	62.36 ± 0.641	58.30 ± 0.050	70.27 ± 0.435
12	63.90 ± 0.975	59.34 ± 0.168	72.24 ± 0.644

n = 3*

TABLE 7c. COMPARATIVE *INVITRO* DRUG RELEASE STUDY OF CEFAZOLIN LOADED BOVINE SERUM ALBUMIN NANOPARTICLE FORMULATIONS F7, F8, F9.

TIME IN HOURS	CUMULATIVE % DRUG RELEASE ± SD		
	F7	F8	F9
0.5	6.35 ± 0.712	14.30 ± 0.712	16.68 ± 0.522
1	14.74 ± 0.727	23.32 ± 1.305	24.91 ± 0.896
1.5	21.81 ± 0.512	28.91 ± 1.448	31.52 ± 1.123
2	26.94 ± 0.544	32.82 ± 1.235	35.92 ± 1.275
3	31.22 ± 0.689	36.26 ± 1.172	39.56 ± 1.056
4	33.77 ± 0.722	38.92 ± 1.395	43.14 ± 0.755
5	36.51 ± 0.930	41.48 ± 1.427	46.50 ± 0.835
6	38.74 ± 1.025	43.81 ± 1.656	49.78 ± 1.032
7	40.58 ± 0.599	46.18 ± 1.589	52.14 ± 0.719
8	42.17 ± 0.799	48.44 ± 1.624	54.53 ± 0.402
9	43.50 ± 0.817	50.59 ± 1.508	56.54 ± 0.411
10	44.70 ± 1.025	52.77 ± 1.483	58.28 ± 0.511
11	45.62 ± 1.048	54.83 ±1.425	59.76 ± 0.825
12	46.69 ± 1.214	56.37 ± 1.138	61.39 ± 1.056

n = 3*

TABLE 7d. COMPARATIVE *INVITRO* DRUG RELEASE STUDY OF CEFAZOLIN LOADED BOVINE SERUM ALBUMIN NANOPARTICLE FORMULATIONS F10, F11, F12.

TIME IN HOURS	CUMULATIVE % DRUG RELEASE ± SD		
	F10	F11	F12
0.5	11.93 ± 0.342	19.89 ± 1.567	15.42 ± 2.061
1	22.84 ± 0.789	33.52 ± 1.421	28.09 ± 1.234
1.5	31.08 ± 0.729	42.98 ± 1.293	36.46 ± 1.020
2	36.16 ± 1.022	49.33 ± 1.111	43.79 ± 0.765
3	40.23 ± 0.886	53.85 ± 1.309	47.02 ± 0.864
4	43.13 ± 0.770	57.90 ± 1.522	49.93 ± 1.092
5	45.66 ± 0.415	61.46 ± 1.713	52.62 ± 1.462
6	47.67 ± 0.599	64.80 ± 1.595	54.64 ± 1.497
7	49.28 ± 0.471	67.63 ± 2.127	56.54 ± 1.697
8	50.77 ± 0.551	70.07 ± 1.997	58.18 ± 1.735
9	51.99 ± 0.741	72.26 ± 1.876	59.69 ± 1.945
10	52.93 ± 0.806	74.05 ± 1.420	60.94 ± 1.831
11	54.15 ± 1.034	75.29 ± 1.497	62.59 ± 1.870
12	55.10 ± 1.197	76.52 ± 1.662	64.26 ± 1.909

n = 3*

TABLE 7e. COMPARATIVE *INVITRO* DRUG RELEASE STUDY OF CEFAZOLIN LOADED BOVINE SERUM ALBUMIN NANOPARTICLES FORMULATIONS F13, F14, F15.

TIME IN HOURS	CUMULATIVE % DRUG RELEASE ± SD		
	F13	F`14	F15
0.5	23.10 ± 1.044	21.28 ± 0.712	24.22 ± 1.099
1	33.17 ± 1.125	31.87 ± 0.727	35.43 ± 1.405
1.5	42.50 ± 1.592	40.19 ± 0.217	41.60 ± 1.483
2	49.11 ± 0.993	46.60 ± 1.418	46.65 ± 2.174
3	54.18 ± 0.614	50.64 ± 1.267	50.69 ± 2.281
4	58.38 ± 0.645	53.92 ± 1.144	53.96 ± 1.968
5	62.23 ± 0.658	56.54 ± 0.986	56.86 ± 1.367
6	65.03 ± 0.323	59.07 ± 0.847	59.39 ± 1.140
7	67.44 ± 0.153	61.62 ± 1.122	61.95 ± 1.304
8	70.01 ± 0.327	63.93 ± 1.078	64.40 ± 0.819
9	72.34 ± 0.071	65.71 ± 0.547	66.19 ± 0.832
10	74.41 ± 0.270	67.63 ± 0.733	67.42 ± 0.516
11	76.35 ± 0.605	69.43 ± 0.746	68.93 ± 0.176
12	78.02 ± 0.796	70.40 ± 0.943	70.58 ± 0.202

n = 3*

TABLE 7f. COMPARATIVE *INVITRO* DRUG RELEASE STUDY OF CEFAZOLIN LOADED BOVINE SERUM ALBUMIN NANOPARTICLES FORMULATIONS F16, F17, F18.

TIME IN HOURS	CUMULATIVE % DRUG RELEASE ± SD		
	F16	F17	F18
0.5	19.75 ± 1.382	13.89 ± 0.860	16.26 ± 1.044
1	30.71 ± 1.917	20.93 ± 0.882	29.79 ± 0.543
1.5	38.03 ± 2.001	24.80 ± 0.903	35.82 ± 0.893
2	42.99 ± 2.677	27.21 ± 1.090	39.76 ± 0.762
3	46.12 ± 2.203	29.25 ± 0.949	42.80 ± 0.761
4	48.85 ± 1.912	31.05 ± 0.689	45.19 ± 0.987
5	50.81 ± 1.614	32.45 ± 0.807	47.47 ± 1.133
6	52.92 ± 1.133	34.15 ± 1.021	49.24 ± 0.609
7	54.92 ± 0.702	35.88 ± 0.982	50.74 ± 0.622
8	56.52 ± 0.479	36.93 ± 1.332	52.53 ± 0.532
9	57.99 ± 0.096	37.85 ± 1.078	53.49 ± 0.451
10	59.75 ± 0.209	39.33 ± 0.989	55.02 ± 0.459
11	60.96 ± 0.120	40.69 ± 0.814	56.27 ± 0.345
12	62.17 ± 0.421	41.78 ± 0.437	57.39 ± 0.688

n = 3*

TABLE 8a. *INVITRO* RELEASE KINETICS OF CEFAZOLIN LOADED BOVINE SERUM ALBUMIN NANOPARTICLES.

Formulation Code	Zero order		First order		Higuchi model		Korsmeyer peppas		Hixon-Crowell	
	R^2	K_0 (h^{-1})	R^2	K_1 (h^{-1})	R^2	K_H ($h^{-1/2}$)	R^2	n	R^2	K_{HC} ($h^{-1/3}$)
F1	0.787	4.009	0.891	-0.03	0.947	15.21	0.935	0.392	0.859	-0.088
F2	0.78	4.089	0.89	-0.031	0.934	15.54	0.901	0.405	0.857	-0.091
F3	0.786	3.965	0.89	-0.029	0.943	15.04	0.926	0.395	0.858	-0.087
F4	0.776	4.097	0.885	-0.031	0.929	15.63	0.903	0.412	0.852	-0.091
F5	0.777	3.967	0.868	-0.028	0.924	15.42	0.911	0.445	0.84	-0.085
F6	0.747	4.484	0.887	-0.038	0.907	16.88	0.88	0.392	0.845	-0.108
F7	0.808	3.302	0.868	-0.02	0.93	13.21	0.896	0.537	0.849	-0.064
F8	0.823	3.599	0.907	-0.024	0.971	13.6	0.964	0.382	0.882	-0.075
F9	0.819	3.977	0.913	-0.029	0.973	15.08	0.976	0.38	0.885	-0.087

TABLE 8b. *INVITRO* RELEASE KINETICS OF CEFAZOLIN LOADED BOVINE SERUM ALBUMIN NANOPARTICLES.

Formulation Code	Zero order		First order		Higuchi model		Korsmeyer peppas		Hixon-Crowell	
	R^2	K_0 (h^{-1})	R^2	K_1 (h^{-1})	R^2	K_H ($h^{-1/2}$)	R^2	n	R^2	K_{HC} ($h^{-1/3}$)
F10	0.739	3.534	0.826	-0.024	0.898	13.5	0.894	0.409	0.798	-0.073
F11	0.764	4.854	0.912	-0.045	0.935	18.26	0.935	0.373	0.869	-0.124
F12	0.733	3.984	0.846	-0.03	0.903	14.98	0.902	0.38	0.811	-0.089
F13	0.775	4.874	0.925	-0.047	0.953	18.19	0.964	0.354	0.883	-0.126
F14	0.751	4.307	0.886	-0.036	0.939	15.88	0.95	0.339	0.845	-0.103
F15	0.737	4.154	0.88	-0.035	0.951	14.93	0.965	0.303	0.837	-0.1
F16	0.712	3.638	0.83	-0.027	0.918	13.11	0.931	0.312	0.793	-0.081
F17	0.746	2.428	0.815	-0.014	0.954	8.699	0.959	0.302	0.793	-0.046
F18	0.699	3.358	0.804	-0.023	0.896	12.15	0.892	0.325	0.771	-0.071

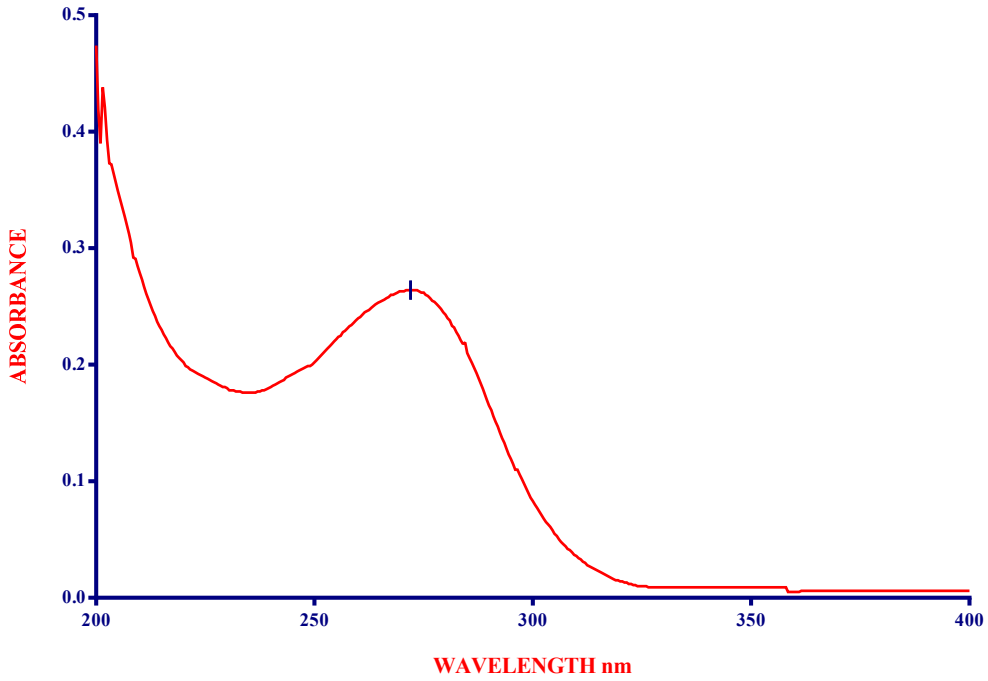


FIGURE 5. DETERMINATION OF ABSORPTION MAXIMUM (λmax) OF CEFAZOLIN (PBS pH 7.4)

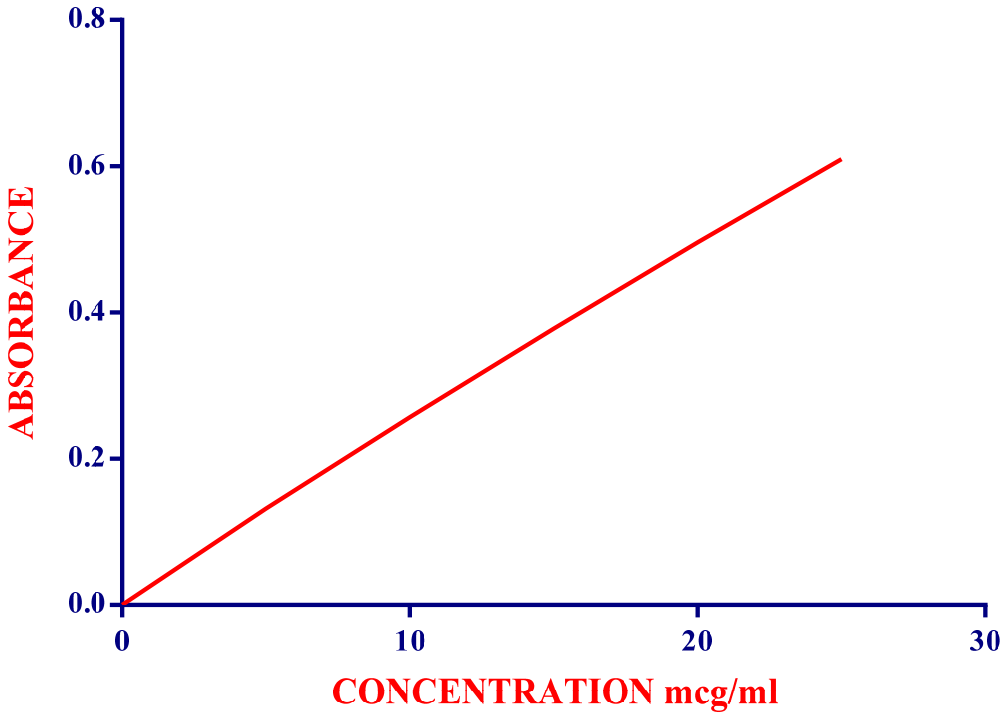


FIGURE 6.CALIBRATION CURVE OF CEFAZOLIN (PBS pH 7.4)

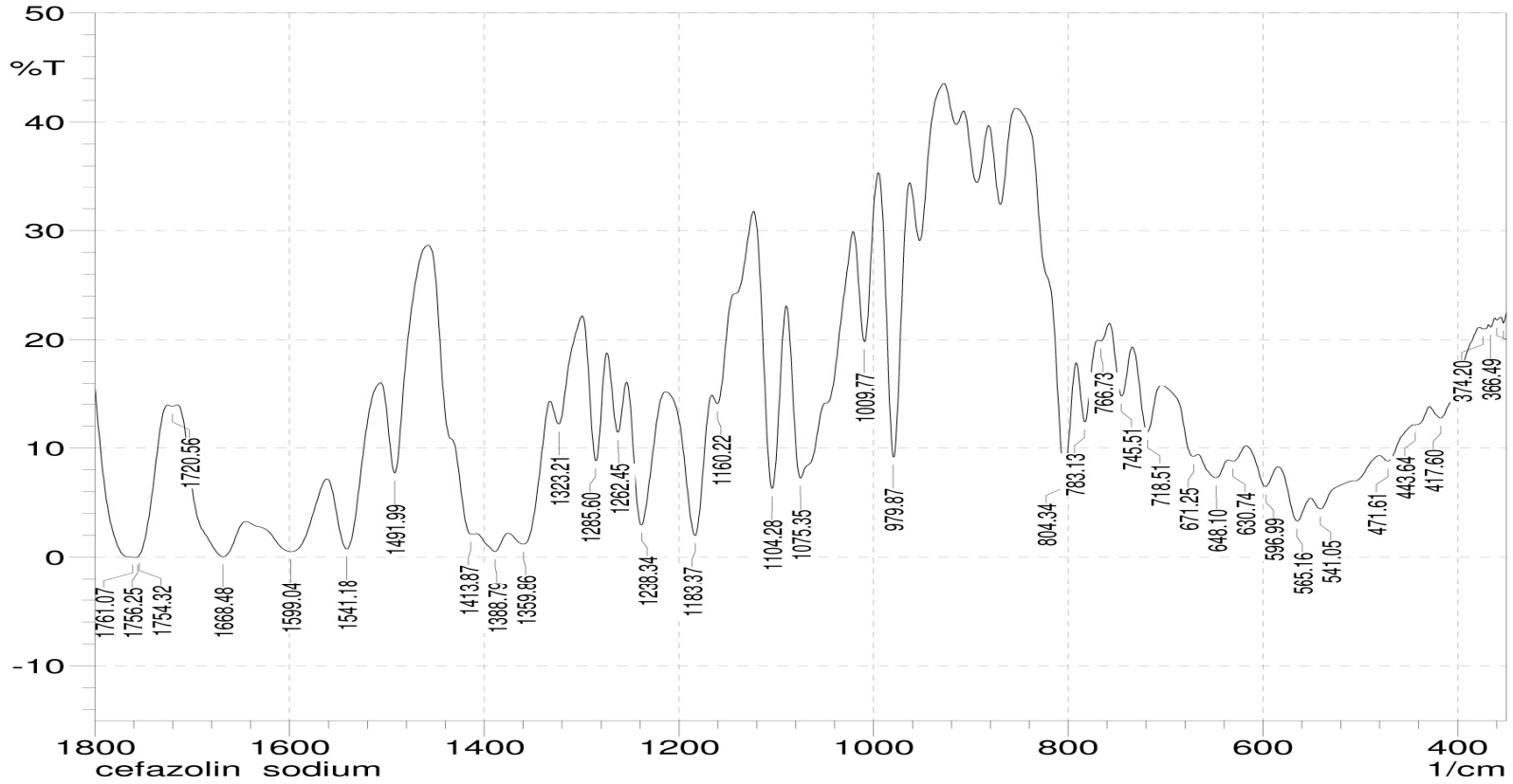


FIGURE 7(a).IR-SPECTRUM OF CEFAZOLIN

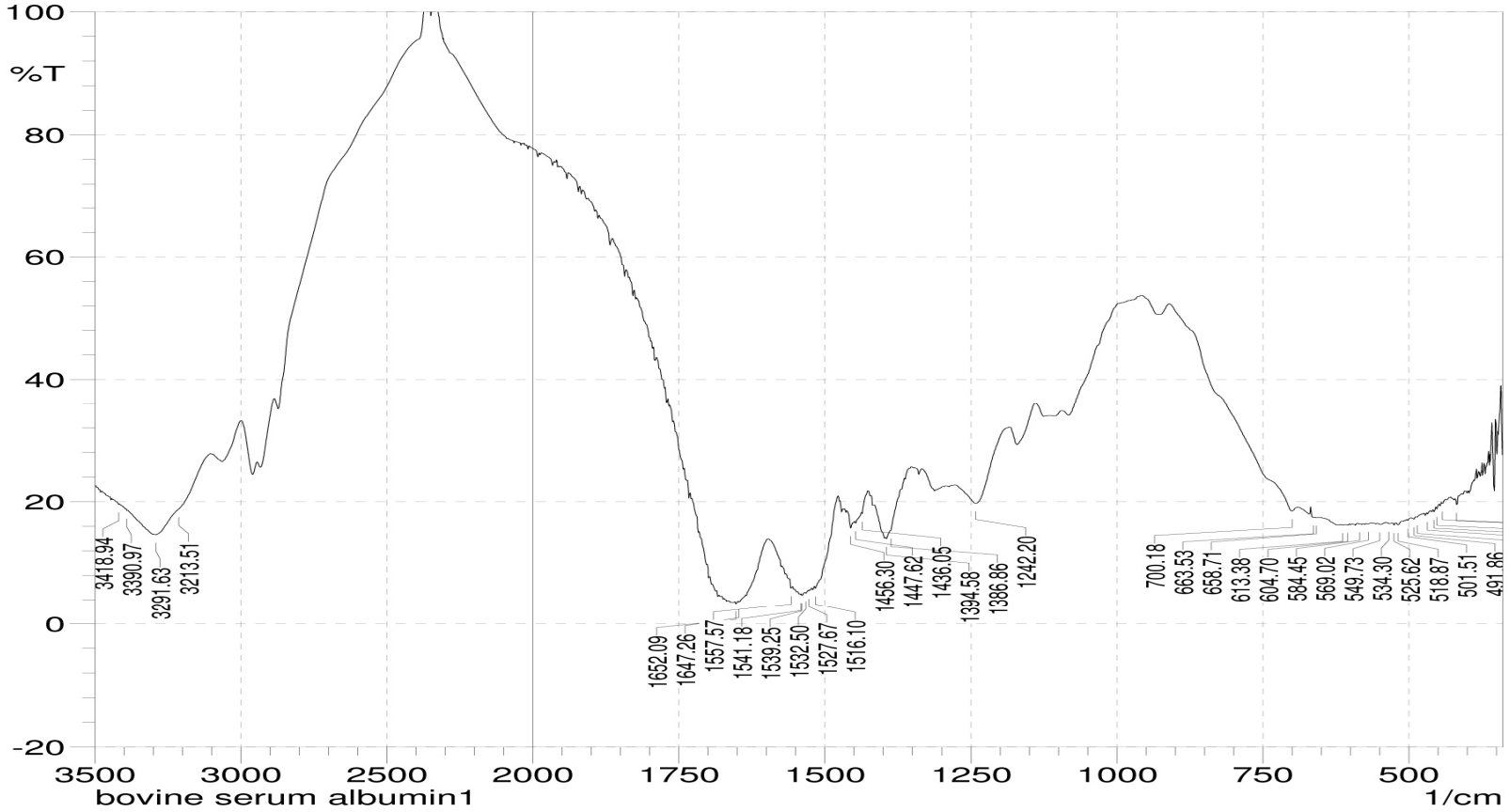


FIGURE 7(b).IR-SPECTRUM OF BOVINE SERUM ALBUMIN

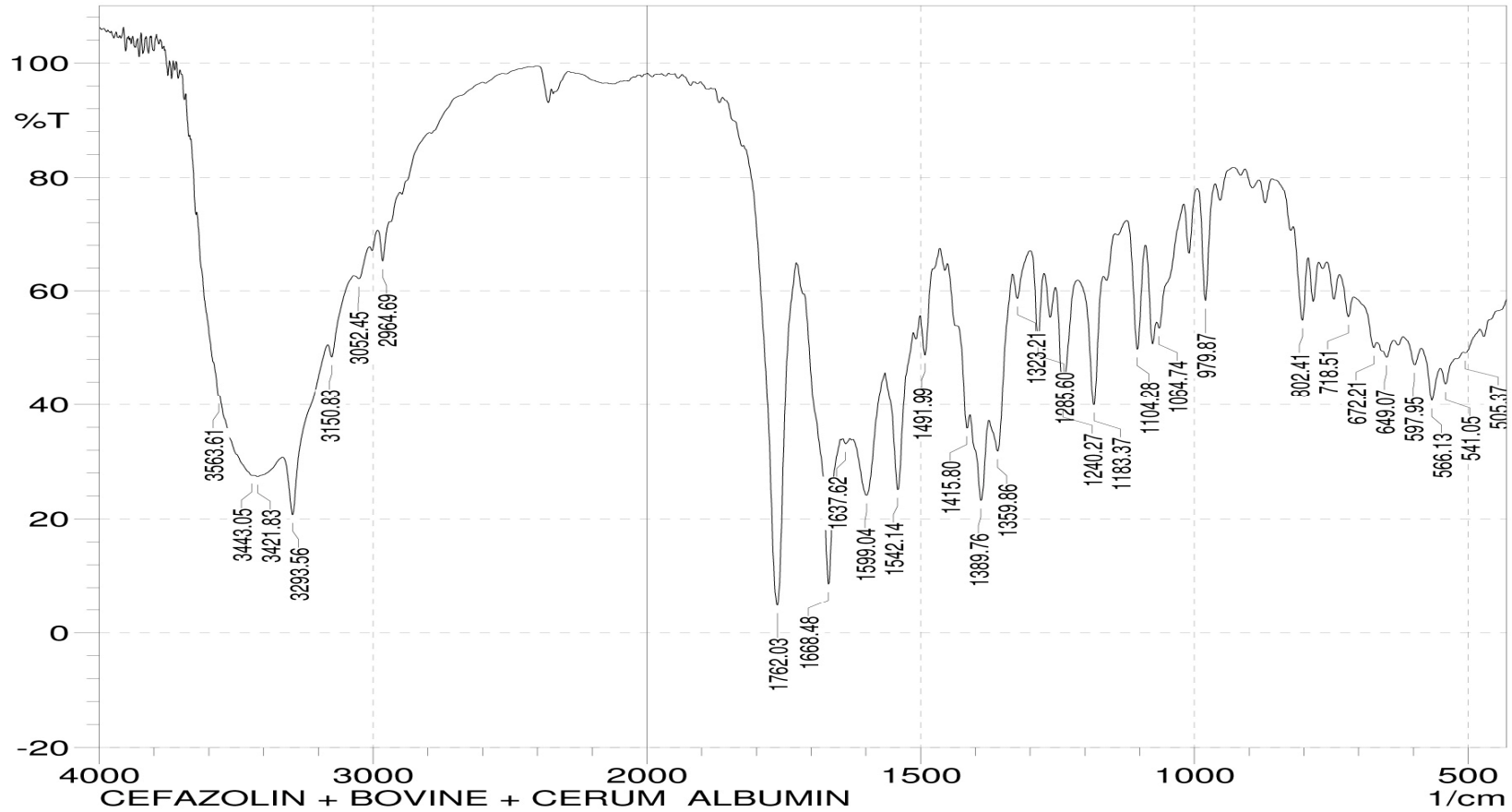


FIGURE 7(c). IR-SPECTRUM OF PHYSICAL MIXTURE

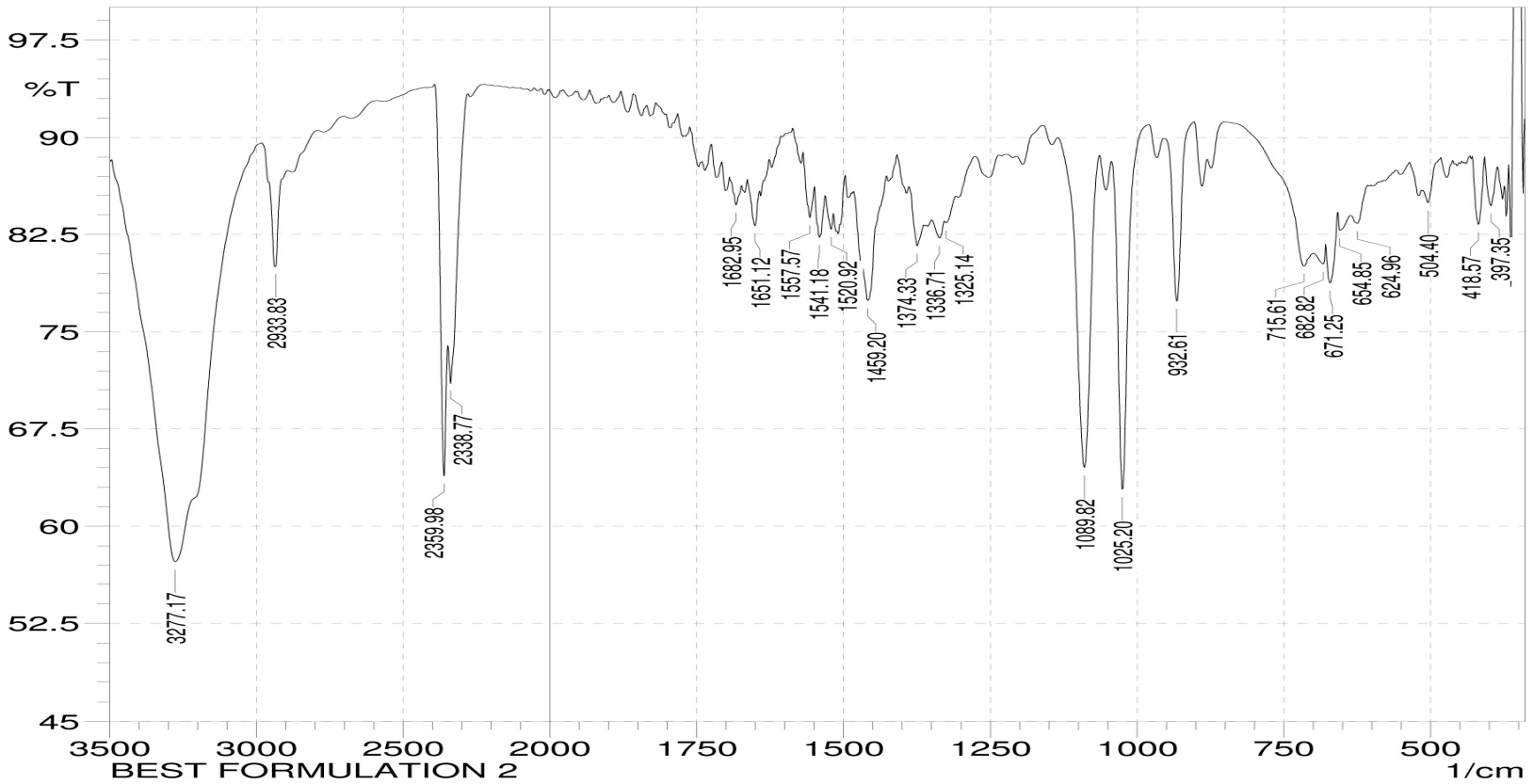


FIGURE 7(d). IR-SPECTRUM OF BEST FORMULATION (F7).

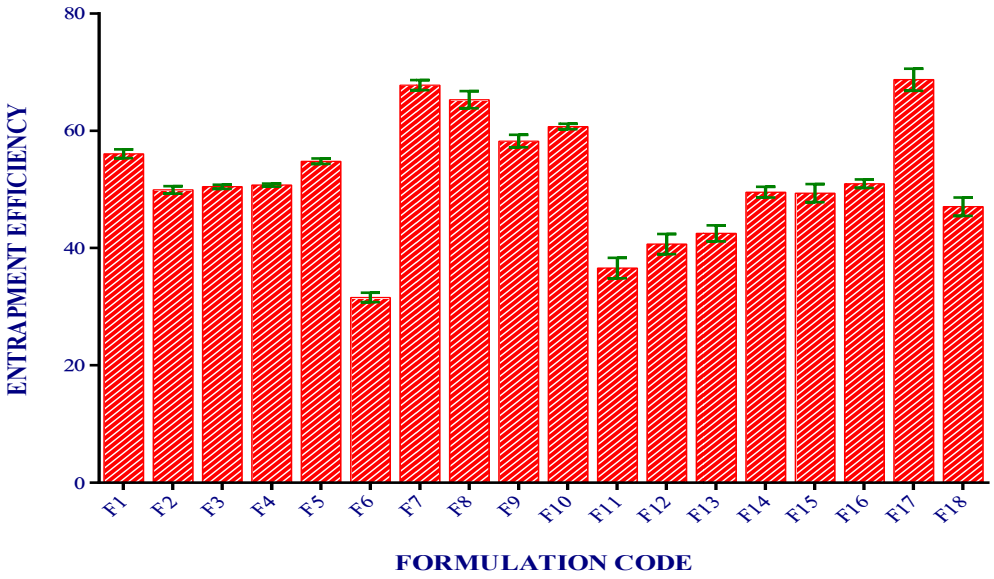


FIGURE 9. COMPARATIVE ENTRAPMENT EFFICIENCY OF CEFAZOLIN LOADED BOVINE SERUM ALBUMIN NANOPARTICLES

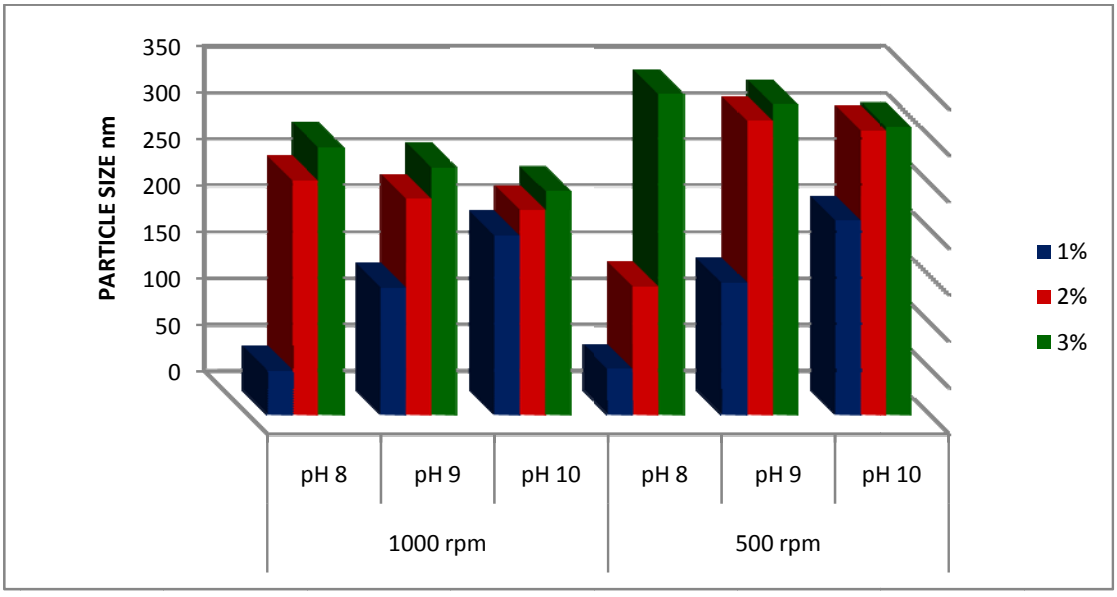
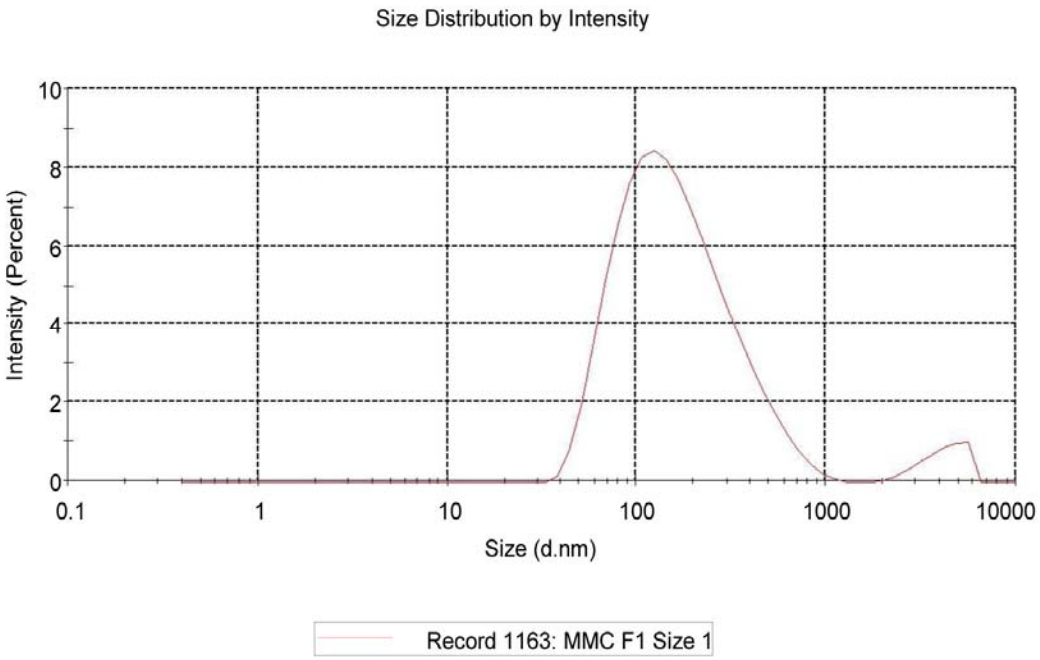


FIGURE 10(a). PARTICLE SIZE OF F1 TO F18



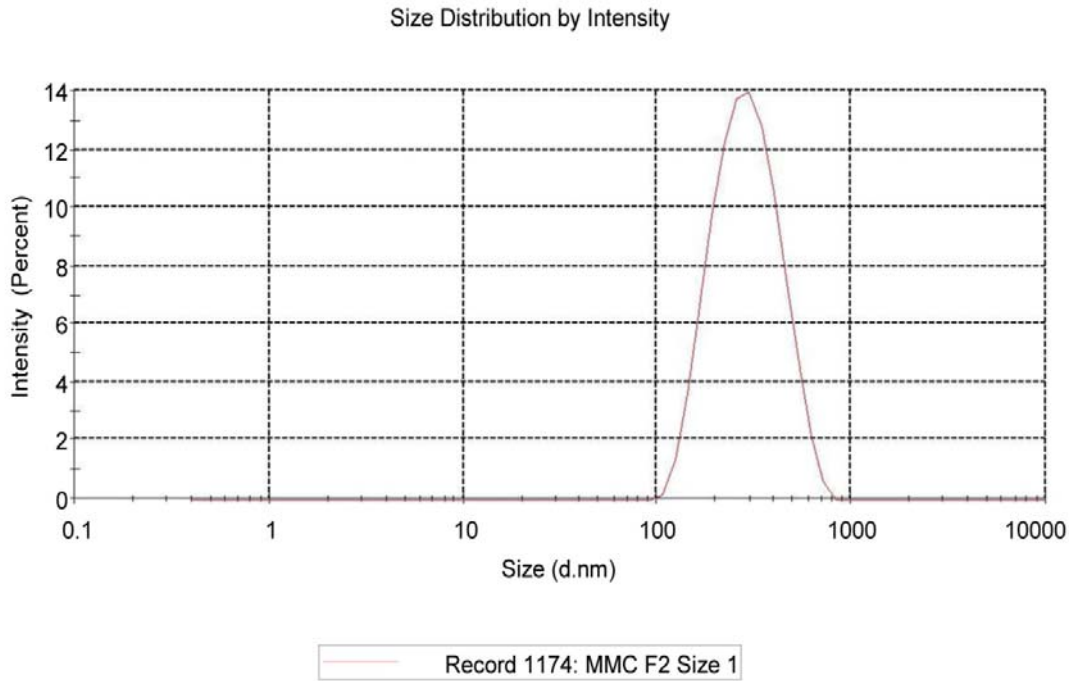
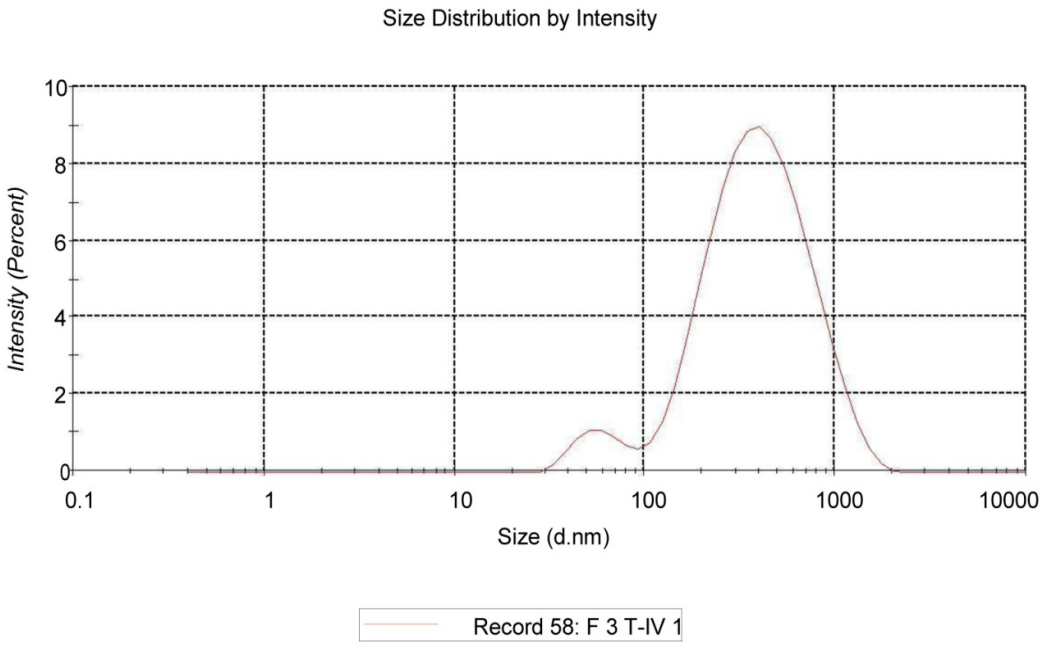


FIGURE 10(b) .PARTICLE SIZE DISTRIBUTION CURVE OF F1 & F2



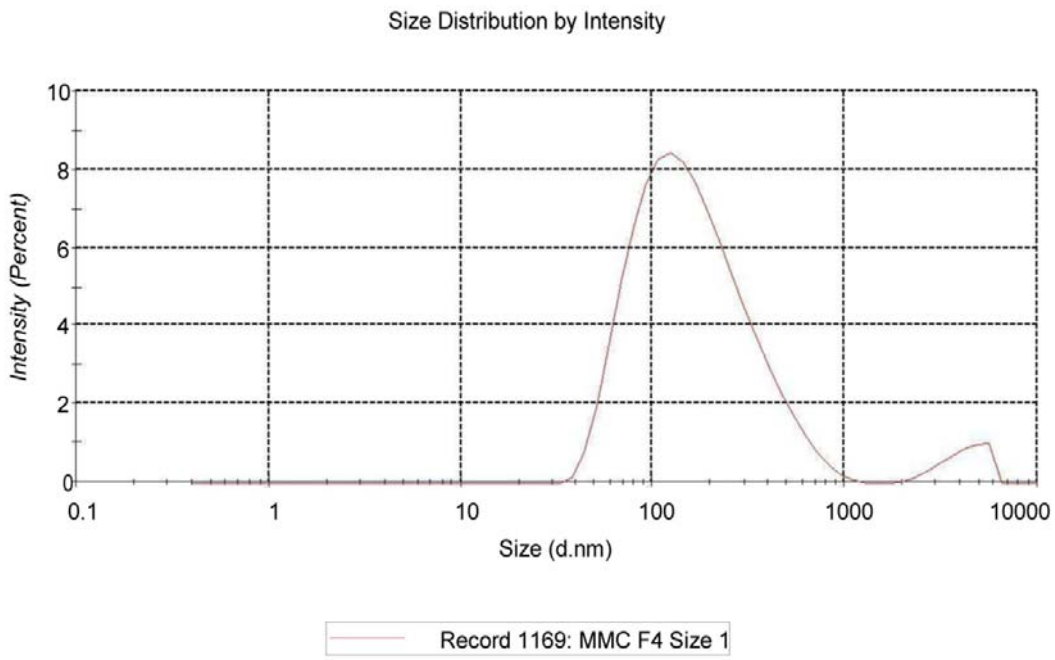
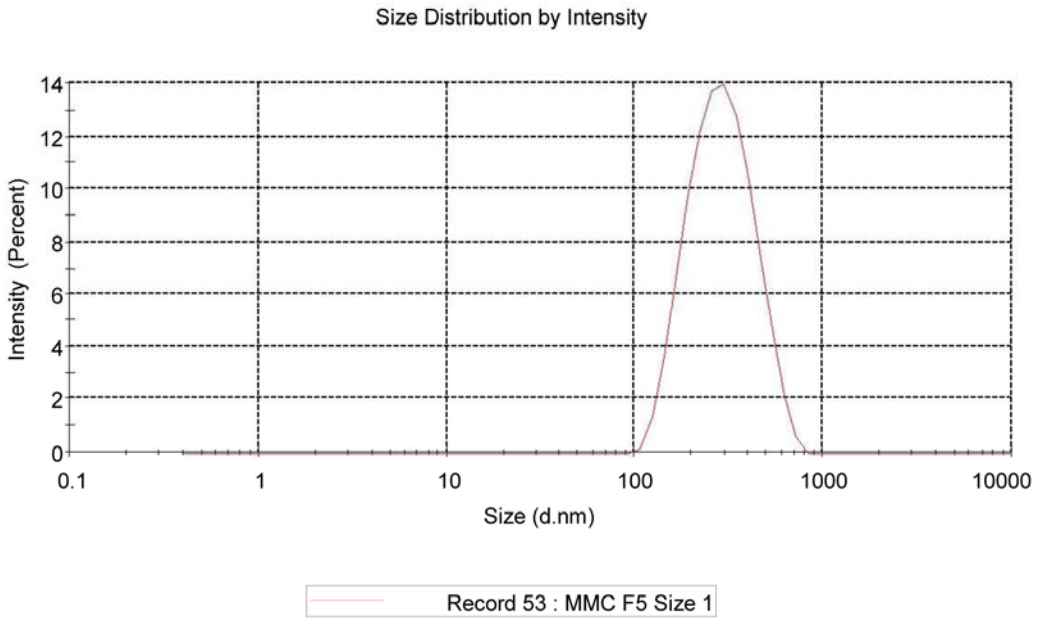


FIGURE 10(c) .PARTICLE SIZE DISTRIBUTION CURVE OF F13& F4



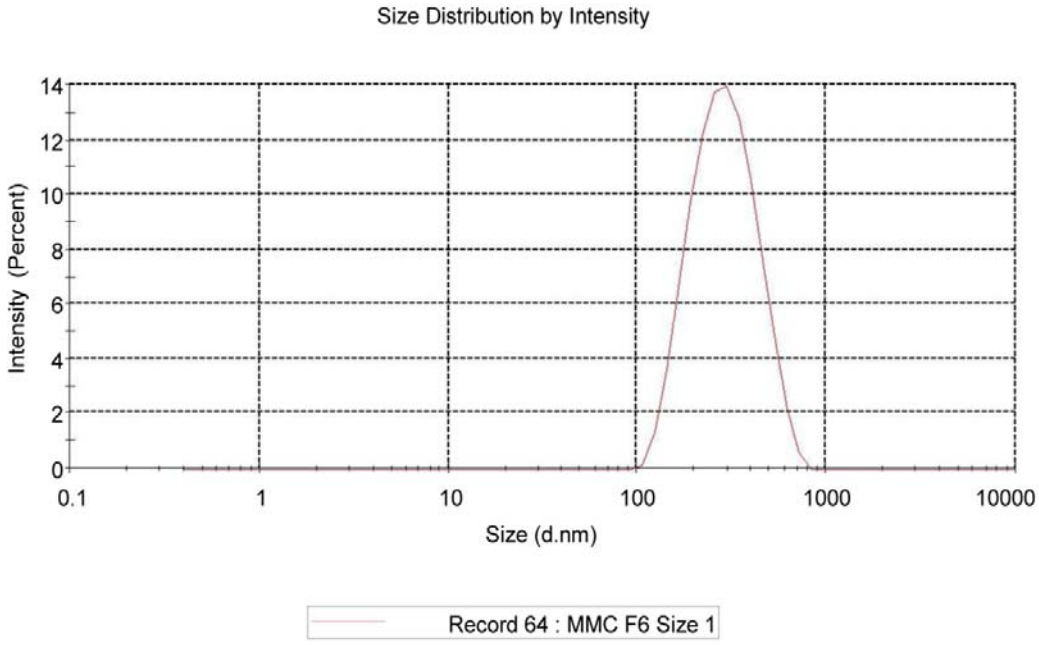
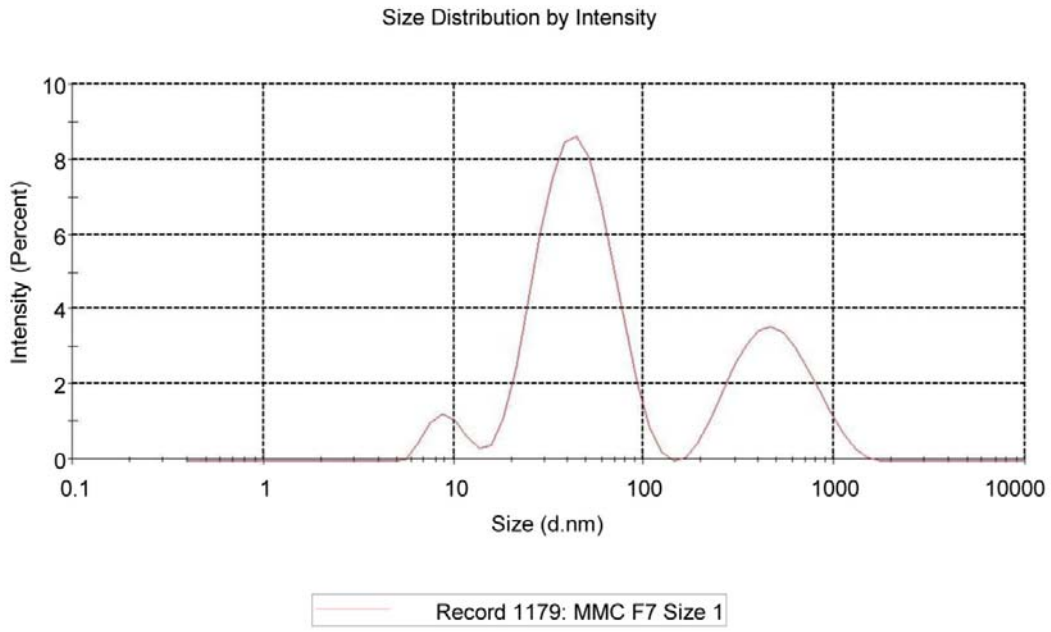


FIGURE 10(d) .PARTICLE SIZE DISTRIBUTION CURVE OF F5 & F6



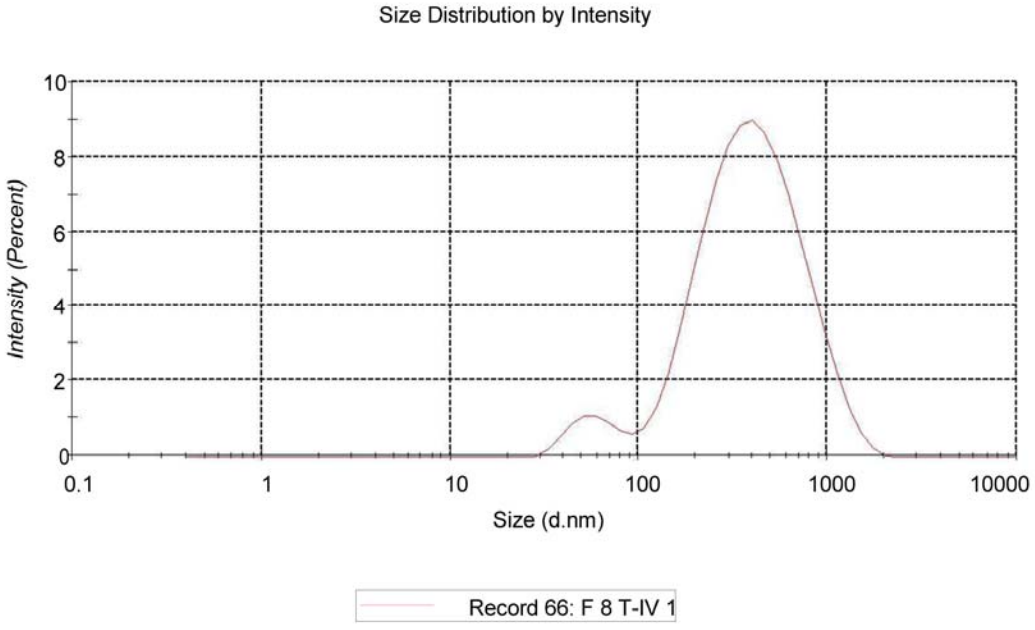
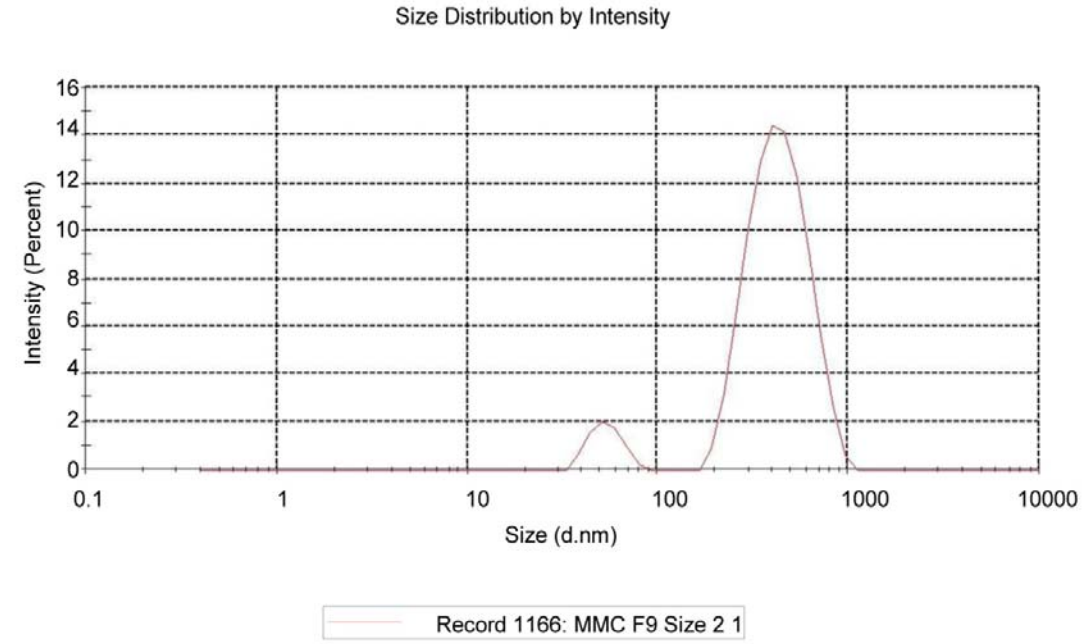


FIGURE 10(e).PARTICLE SIZE DISTRIBUTION CURVE OF F7 & F8



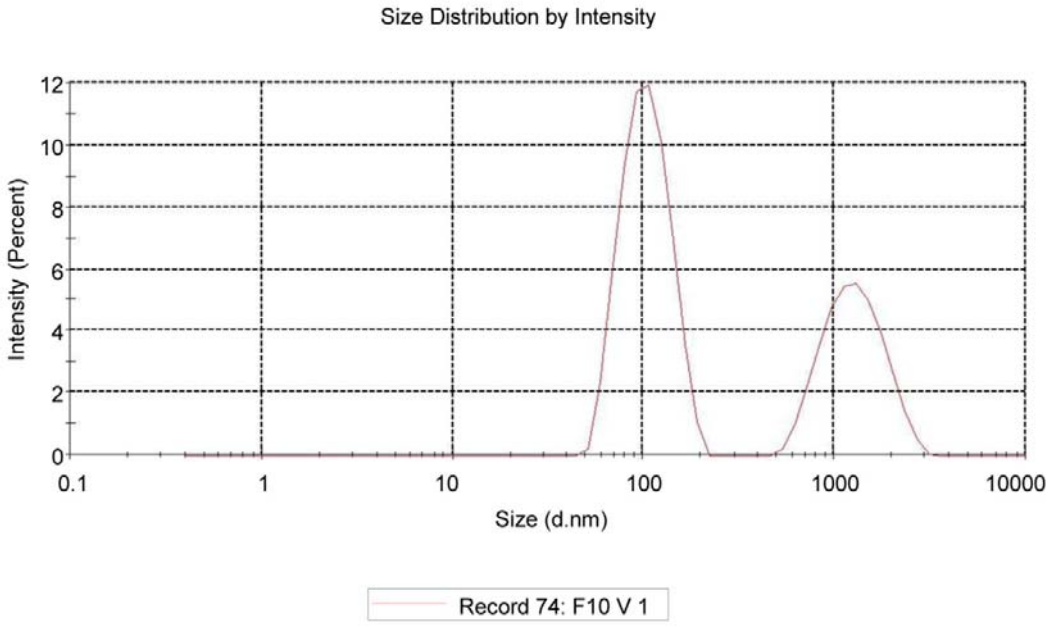
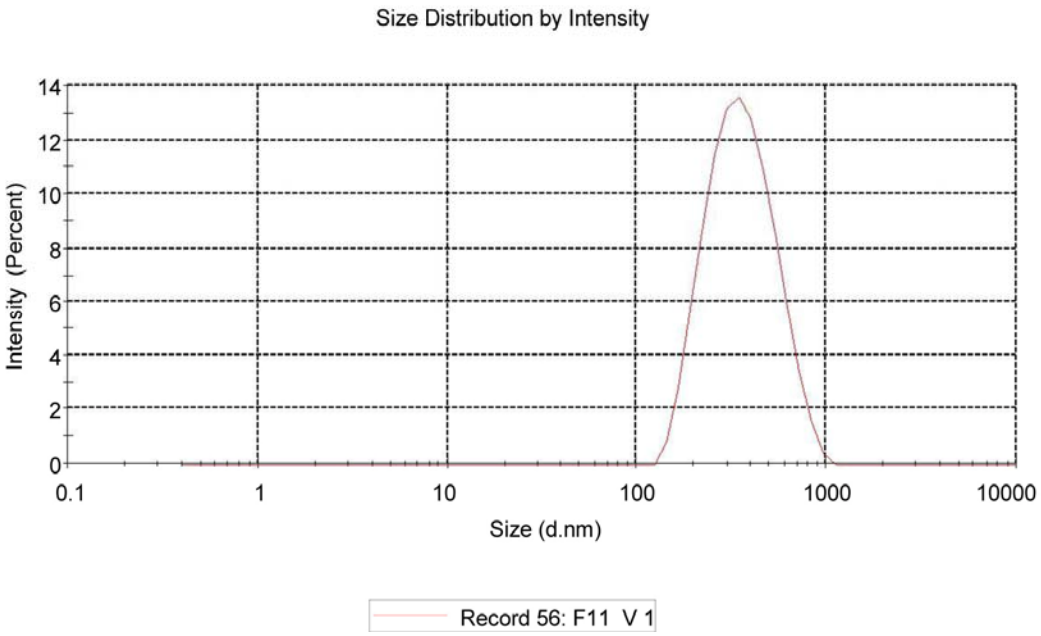


FIGURE 10(f).PARTICLE SIZE DISTRIBUTION CURVE OF F9 & F10



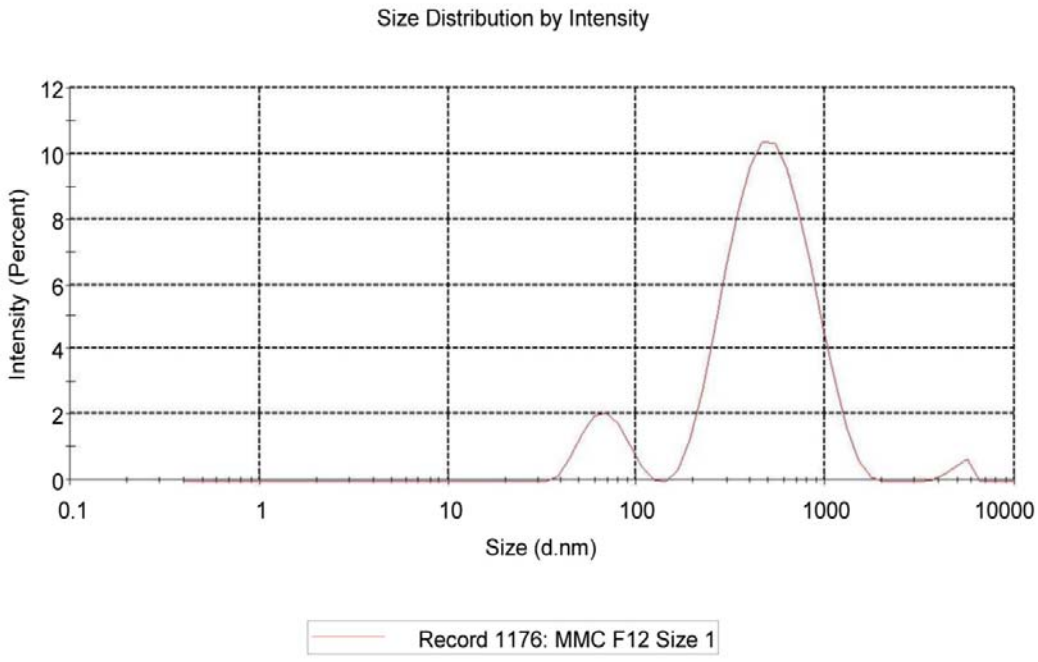
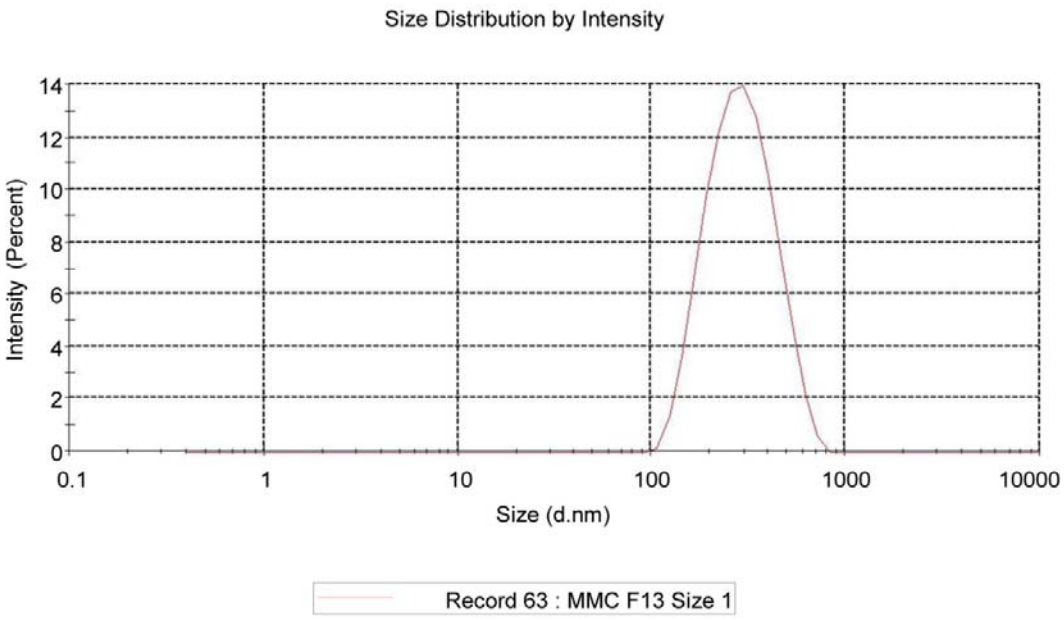


FIGURE 10(g).PARTICLE SIZE DISTRIBUTION CURVE OF F11 & F12



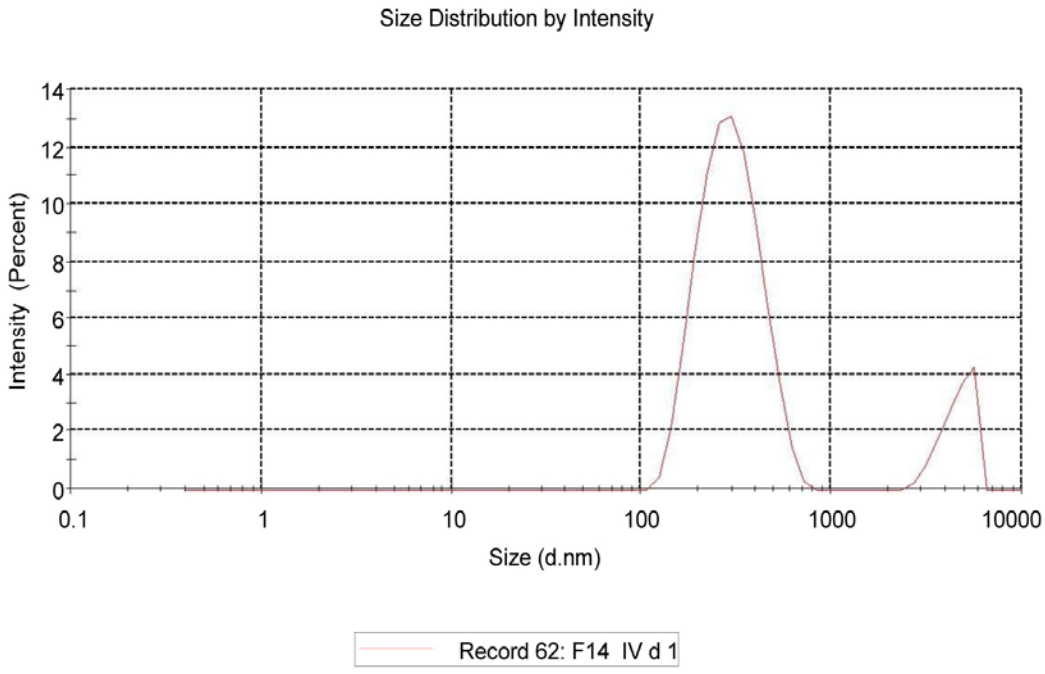
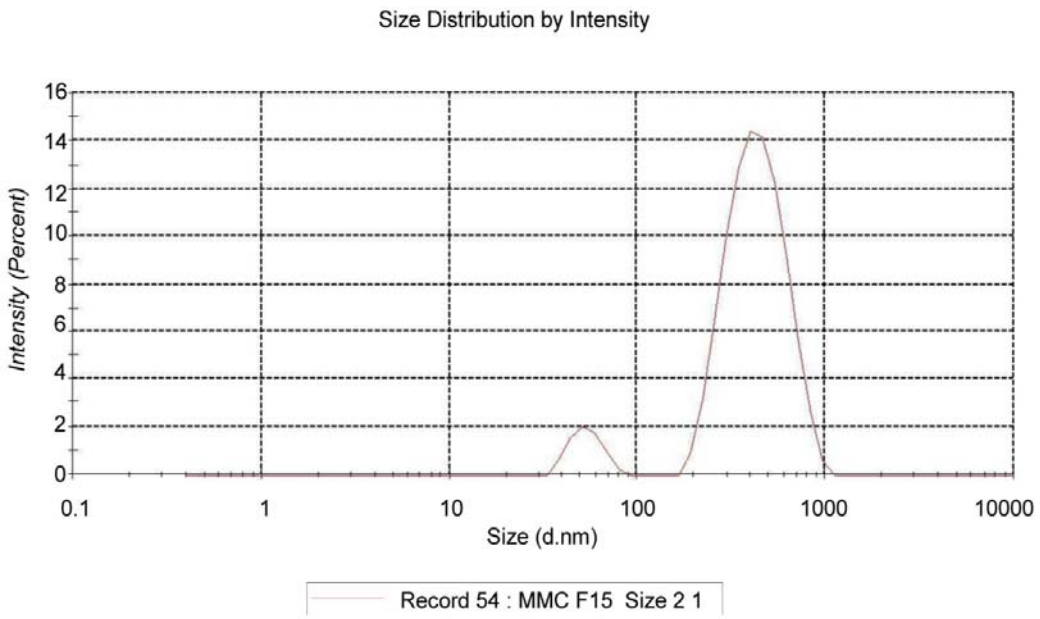


FIGURE 10(h).PARTICLE SIZE DISTRIBUTION CURVE OF F13 & F14



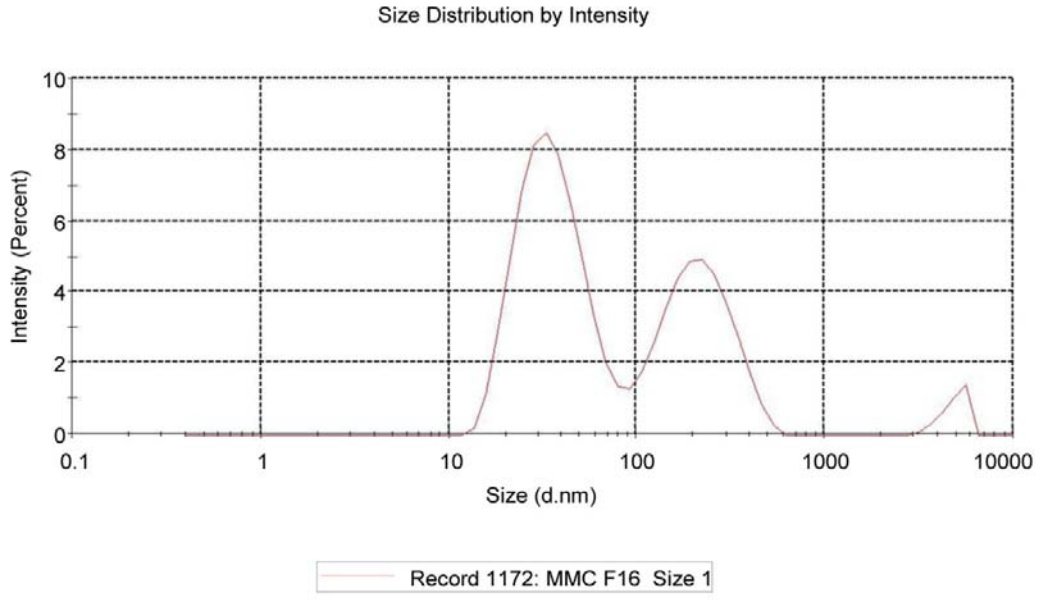
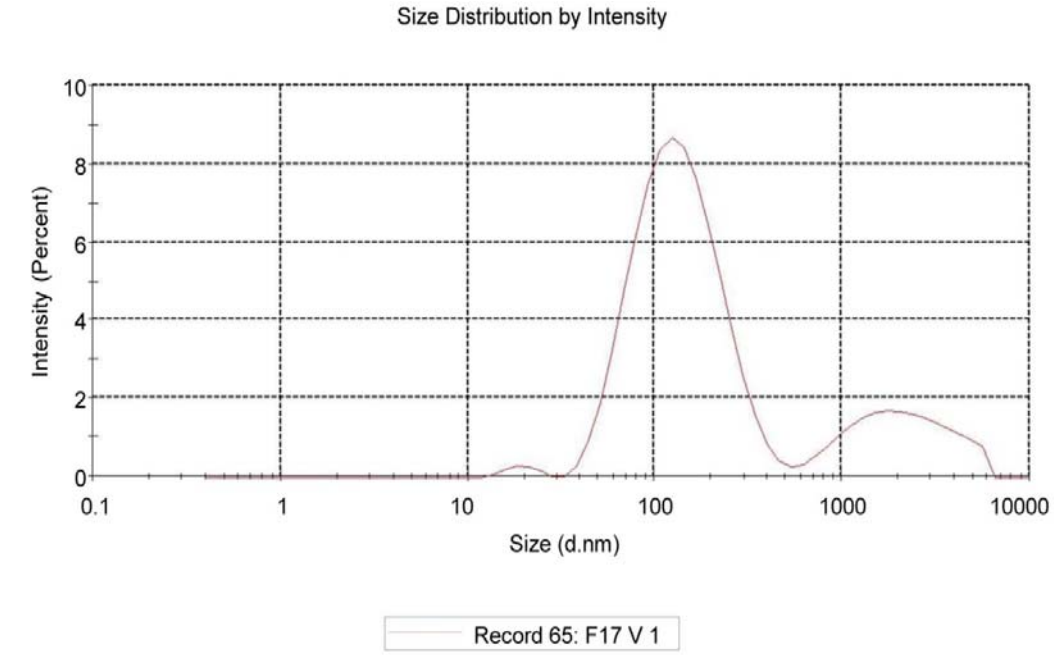


FIGURE 10(i).PARTICLE SIZE DISTRIBUTION CURVE OF F1 5& F16



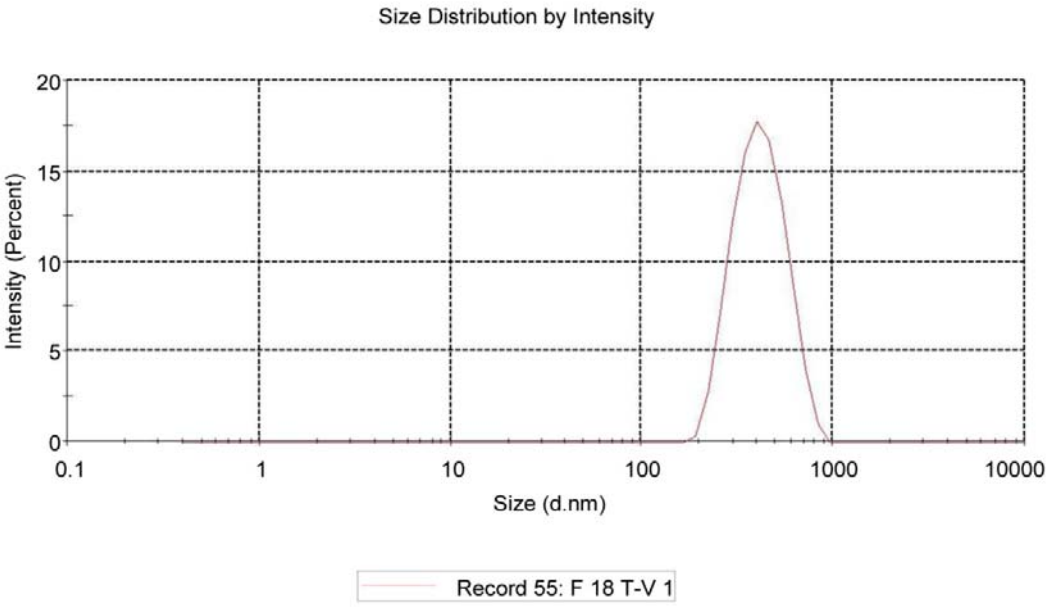
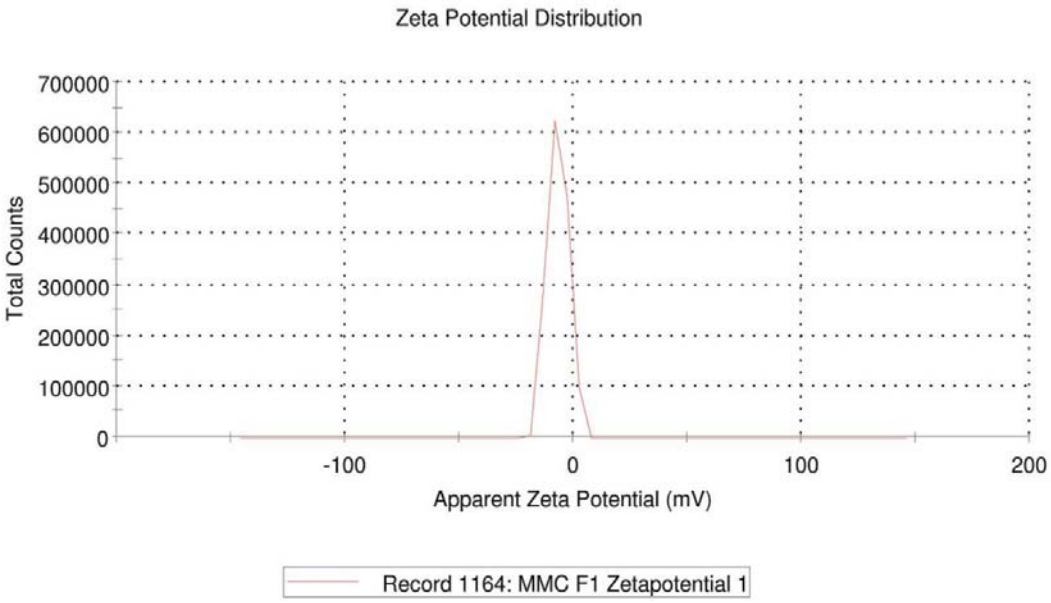


FIGURE 10(j).PARTICLE SIZE DISTRIBUTION CURVE OF F17 & F18



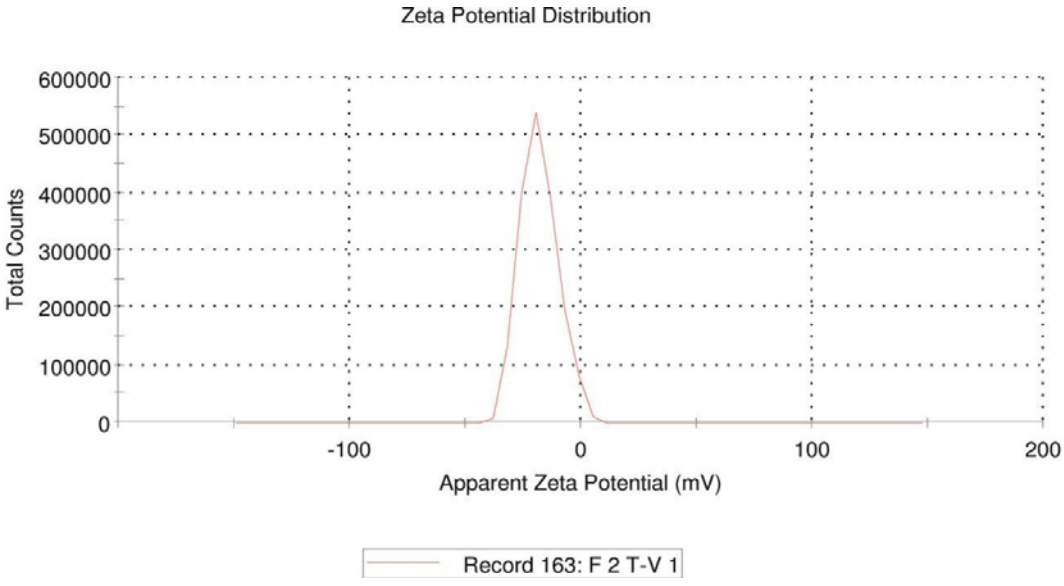
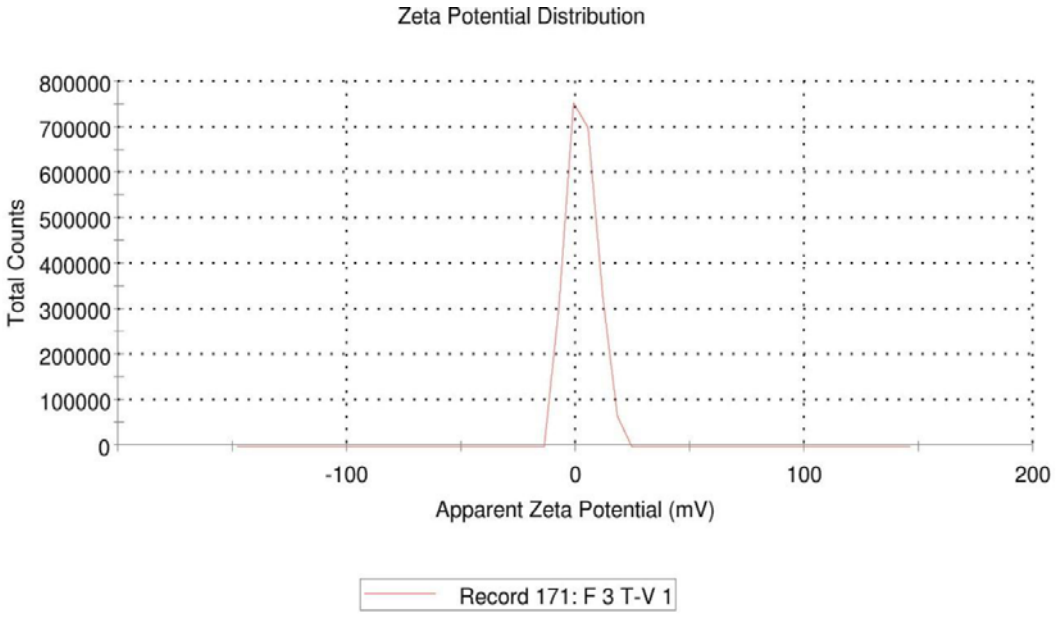


FIGURE 11(a).ZETAPOTENTIAL OF FORMULATION F1& F2



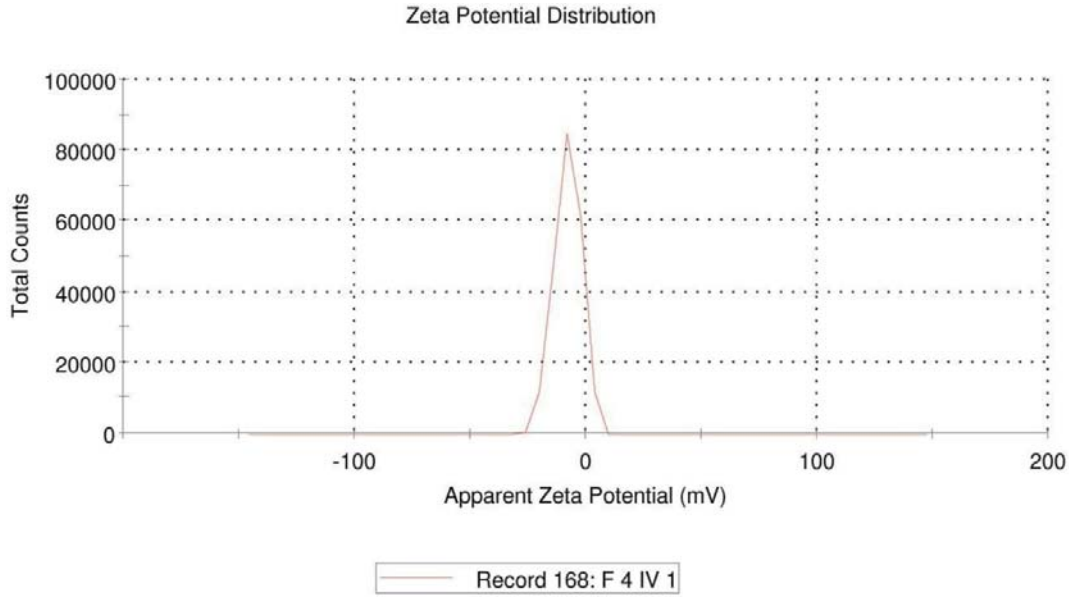
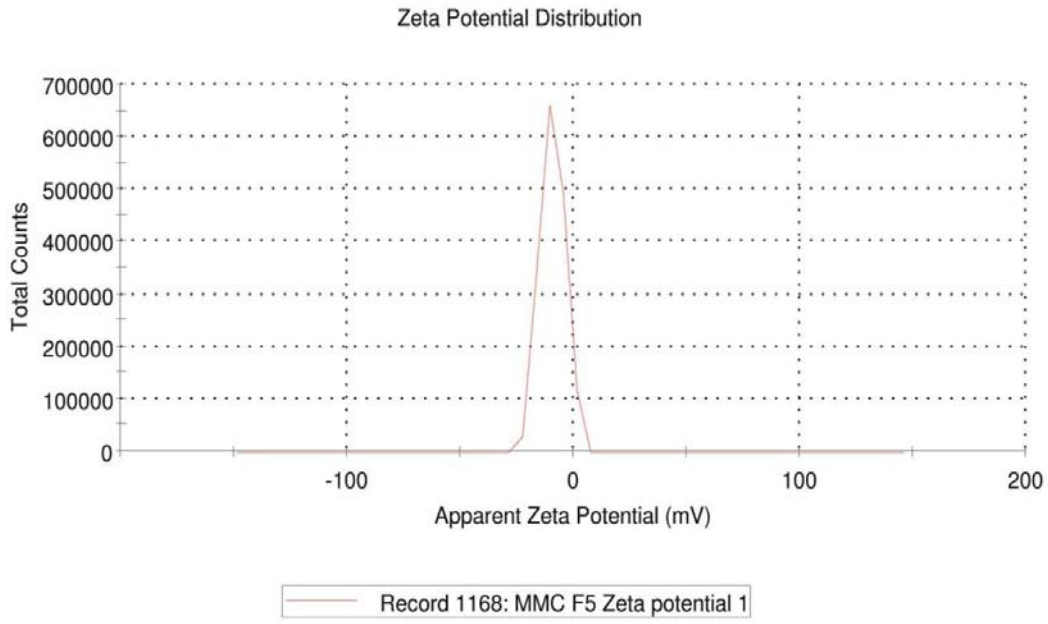


FIGURE 11(b).ZETAPOTENTIAL OF FORMULATION F3& F4



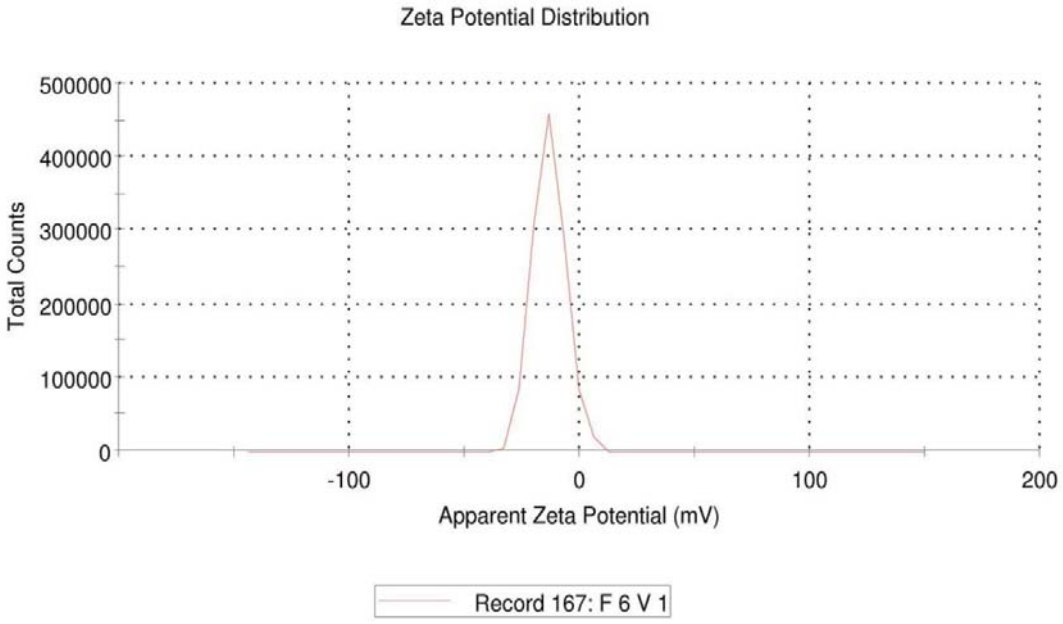
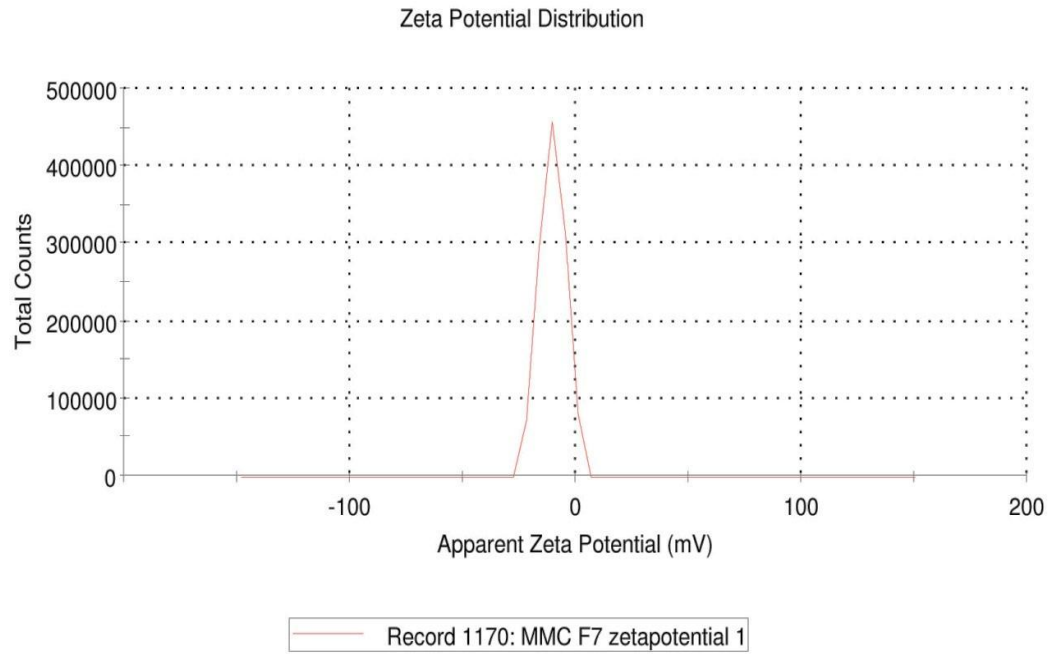


FIGURE 11(c).ZETAPOTENTIAL OF FORMULATION F5& F6



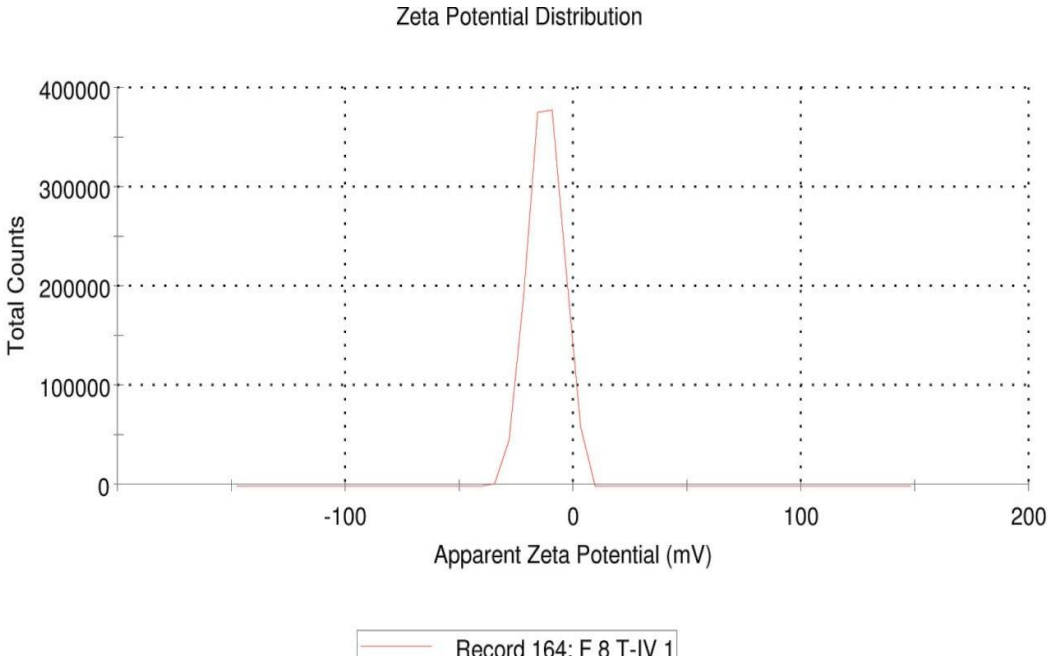
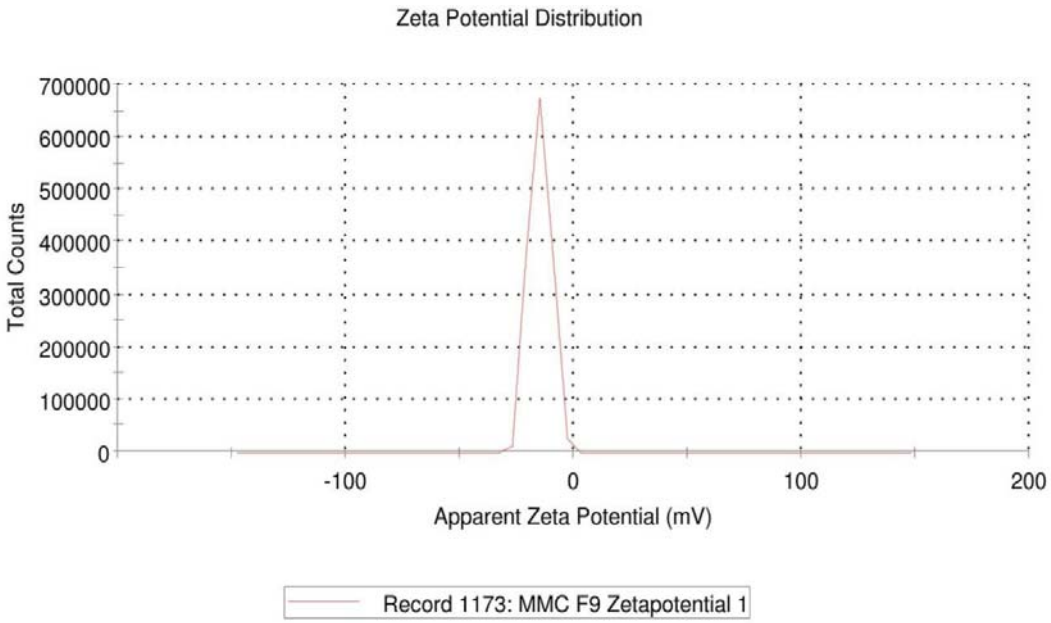


FIGURE 11(d).ZETAPOTENTIAL OF FORMULATION F7& F8



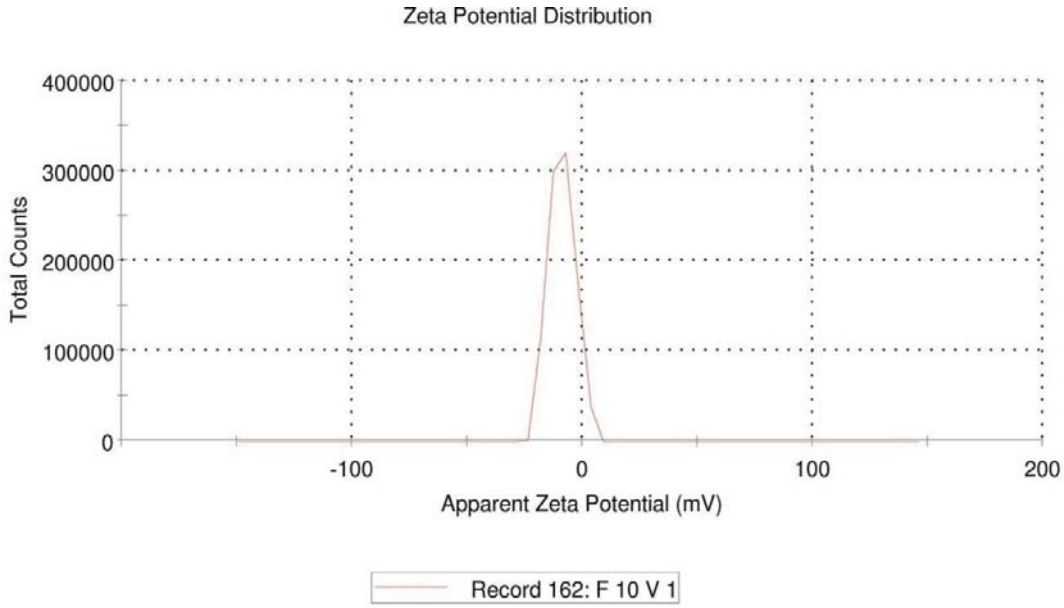
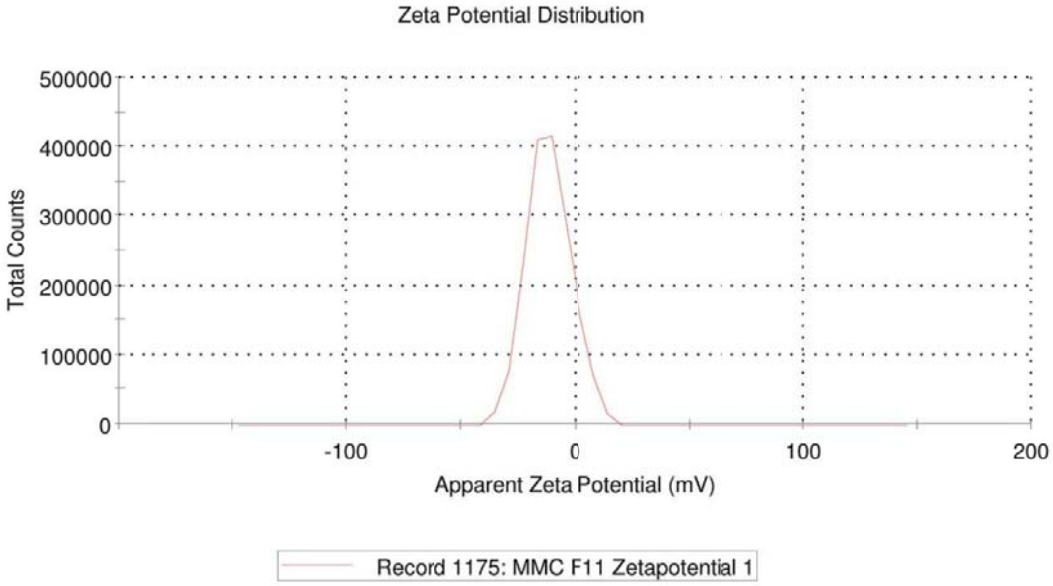


FIGURE 11(e).ZETAPOTENTIAL OF FORMULATION F9& F10



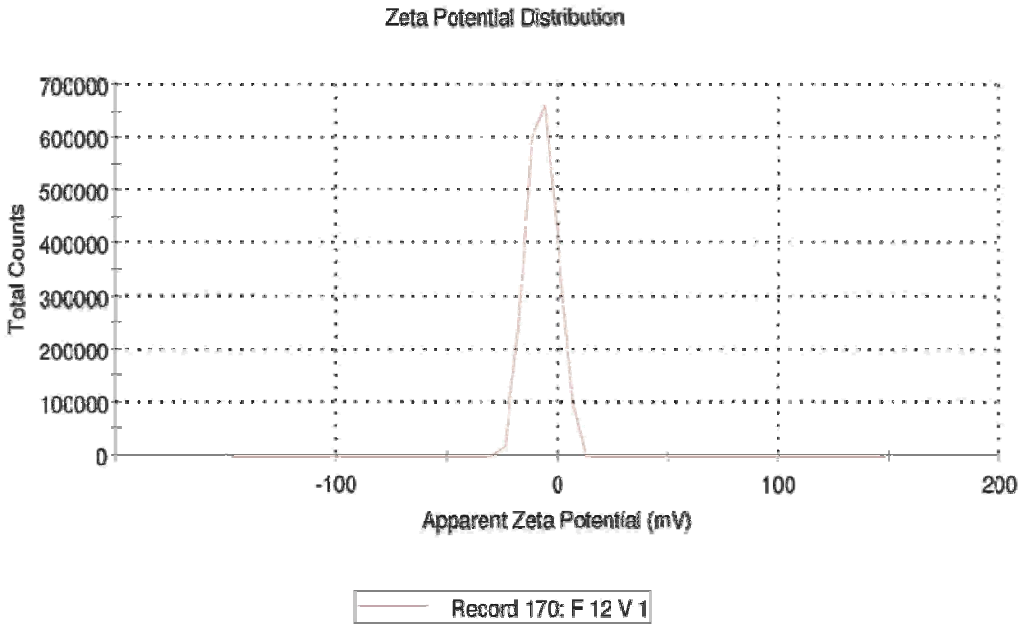
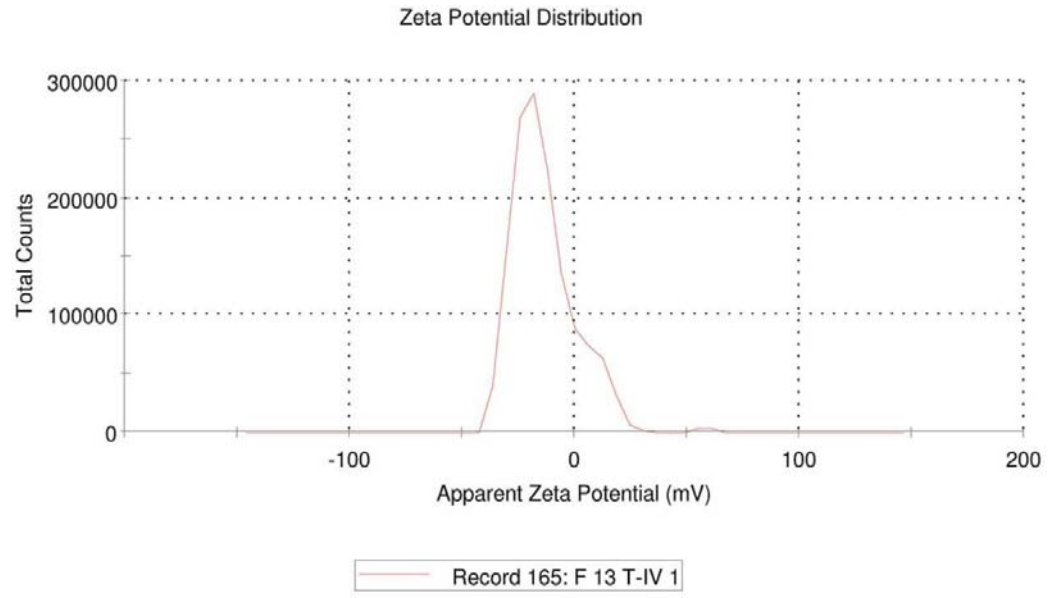


FIGURE 11(f).ZETAPOTENTIAL OF FORMULATION F11& F12



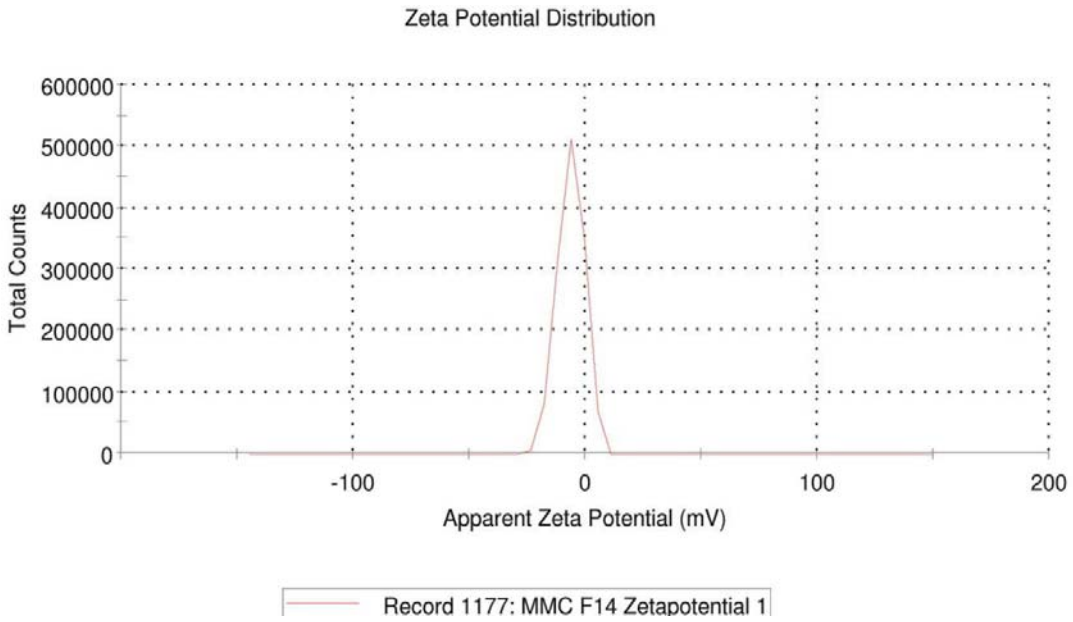
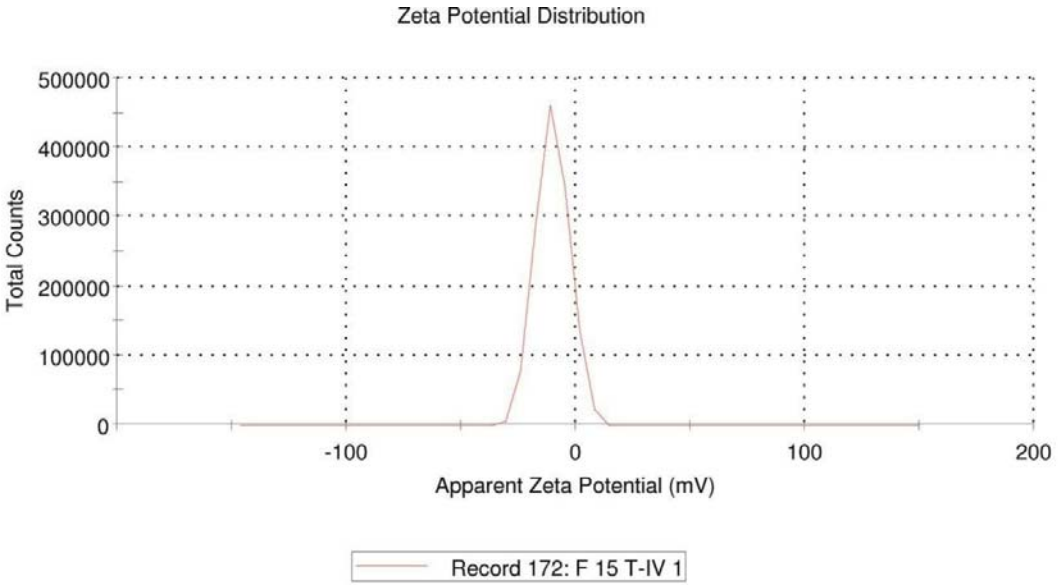


FIGURE 11(g).ZETAPOTENTIAL OF FORMULATION F13& F14



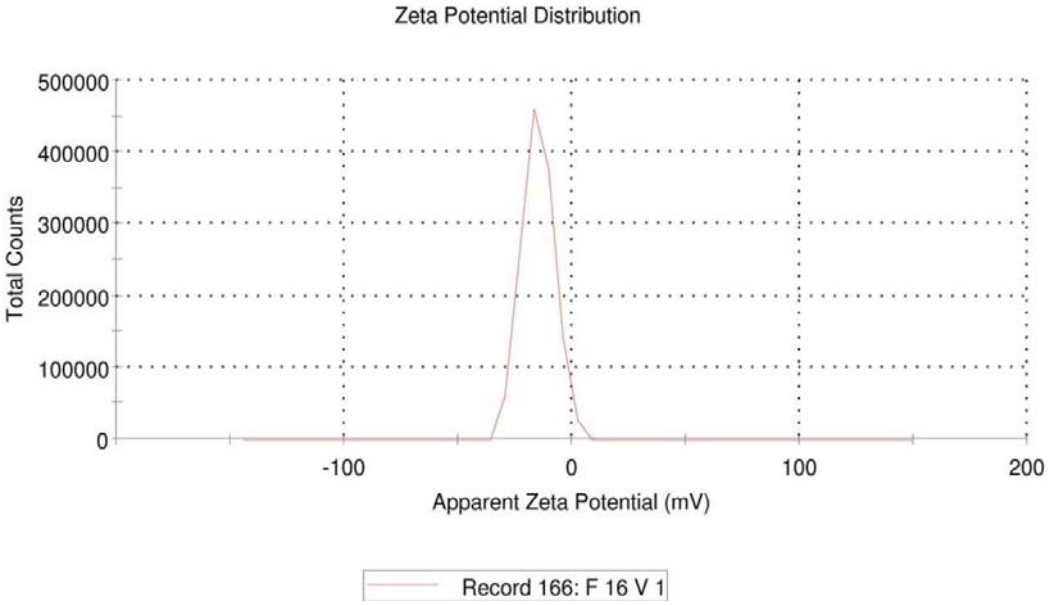
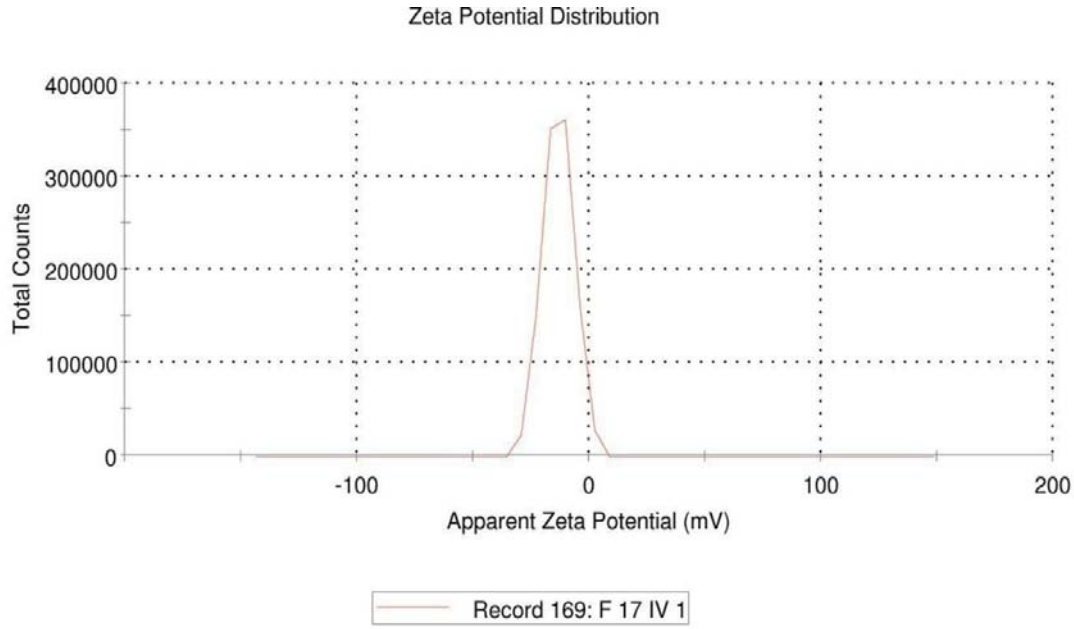


FIGURE 11(h).ZETAPOTENTIAL OF FORMULATION F15& F16



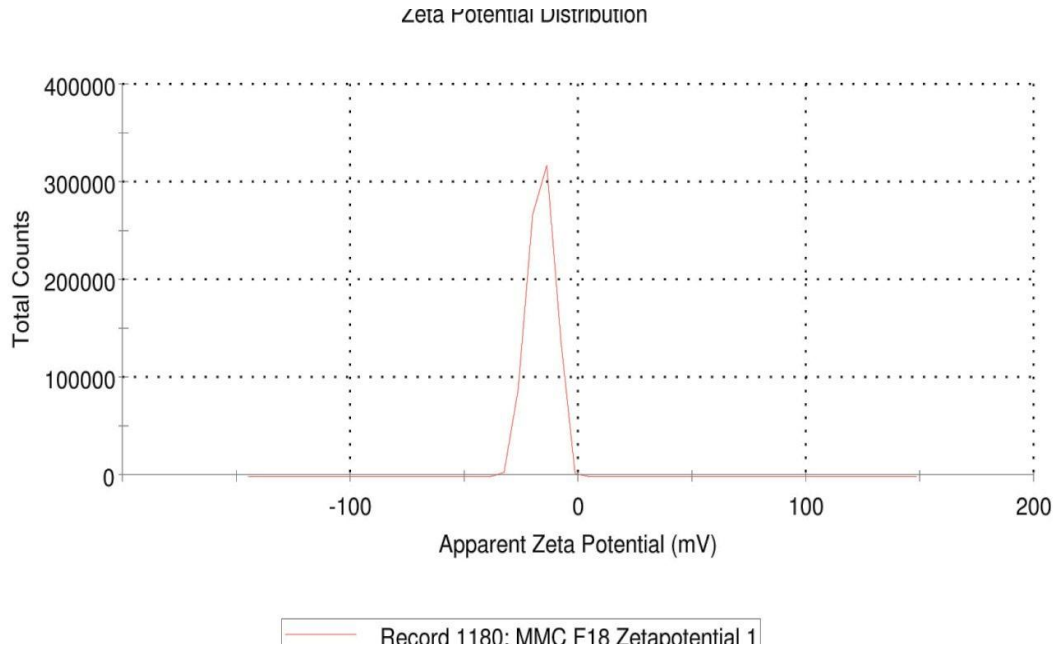


FIGURE 11(i).ZETAPOTENTIAL OF FORMULATION F17& F18

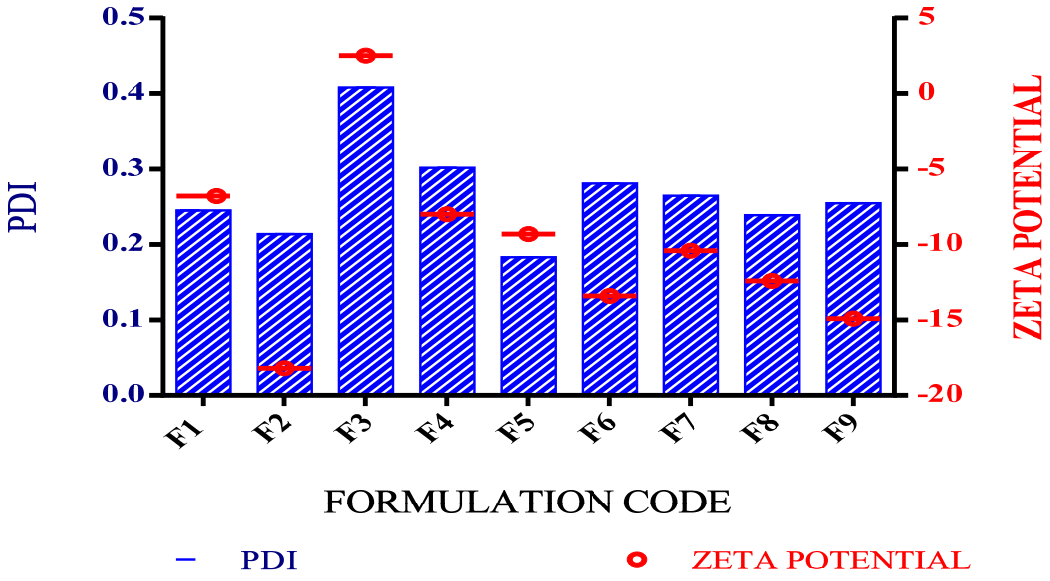


FIGURE 12(a). PDI & ZETA POTENTIAL OF F1 – F9

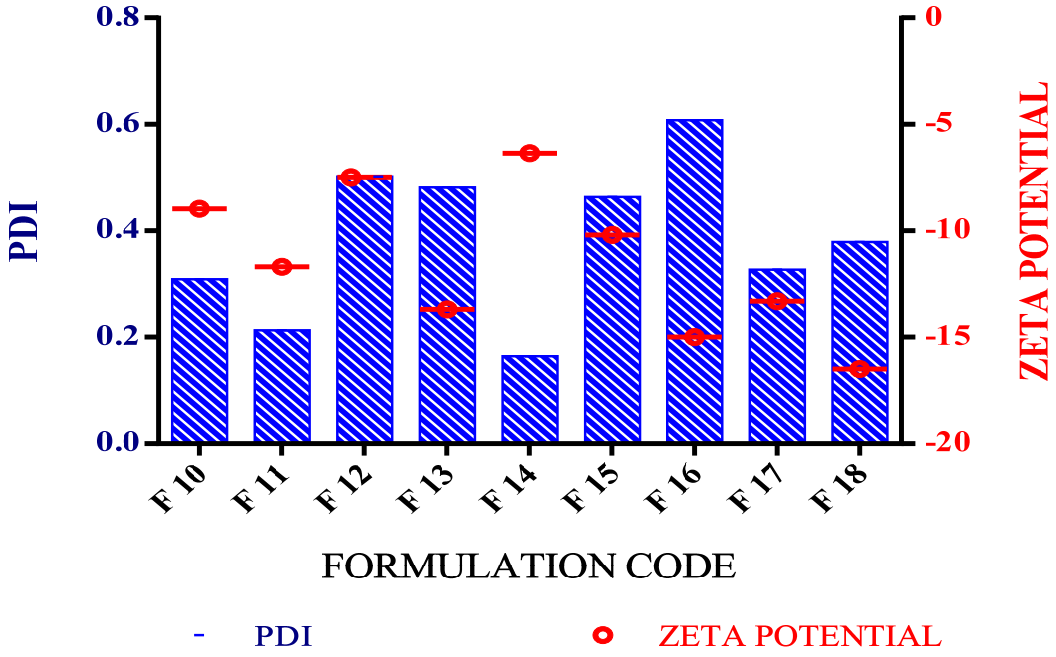


FIGURE 12(b). PDI & ZETA POTENTIAL OF F10 – F18

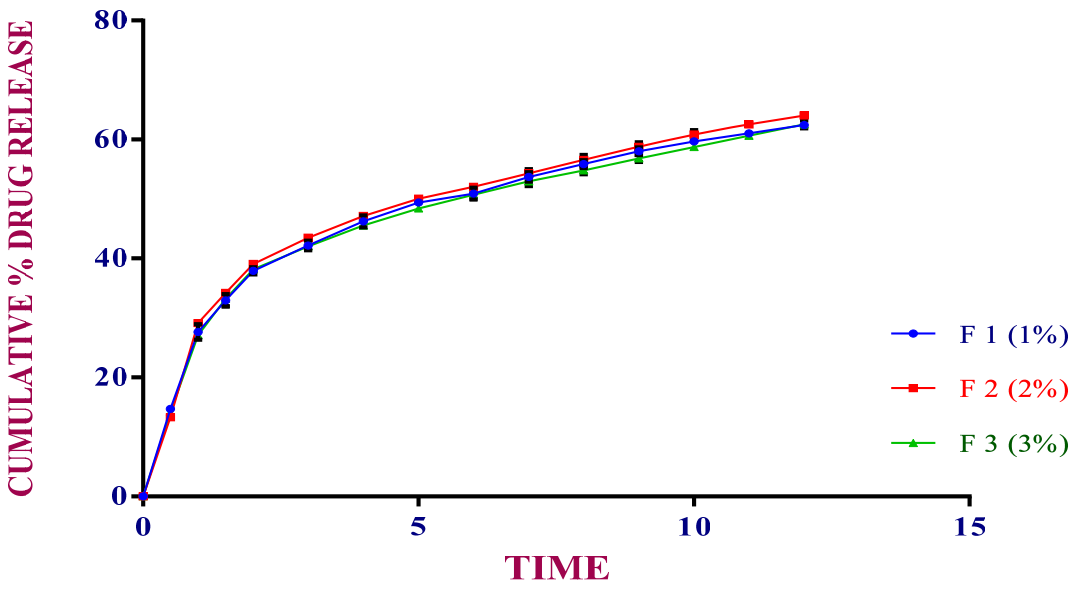


FIGURE 13(a).COMPARATIVE *INVITRO* DRUG RELEASE STUDY OF CEFAZOLIN LOADED BOVINE SERUM ALBUMIN NANOPARTICLES PREPARED AT 1000 RPM IN pH 9.

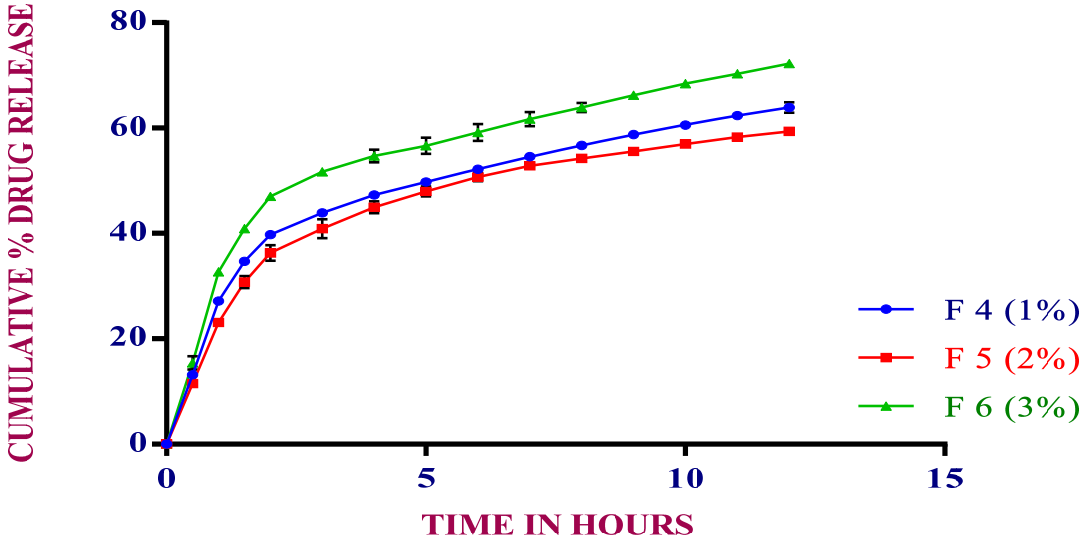


FIGURE 13(b).COMPARATIVE *INVITRO* DRUG RELEASE STUDY OF CEFAZOLIN LOADED BOVINE SERUM ALBUMIN NANOPARTICLES PREPARED AT 1000 RPM IN pH 10.

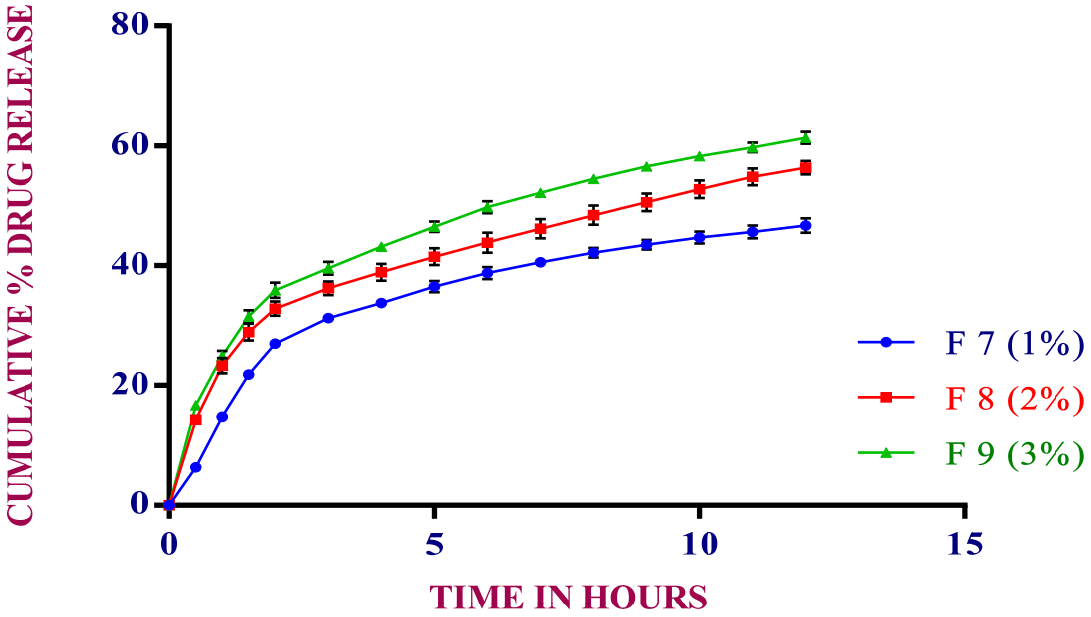


FIGURE 13(c).COMPARATIVE *INVITRO* DRUG RELEASE STUDY OF CEFAZOLIN LOADED BOVINE SERUM ALBUMIN NANOPARTICLES PREPARED AT 1000 RPM IN pH 8.

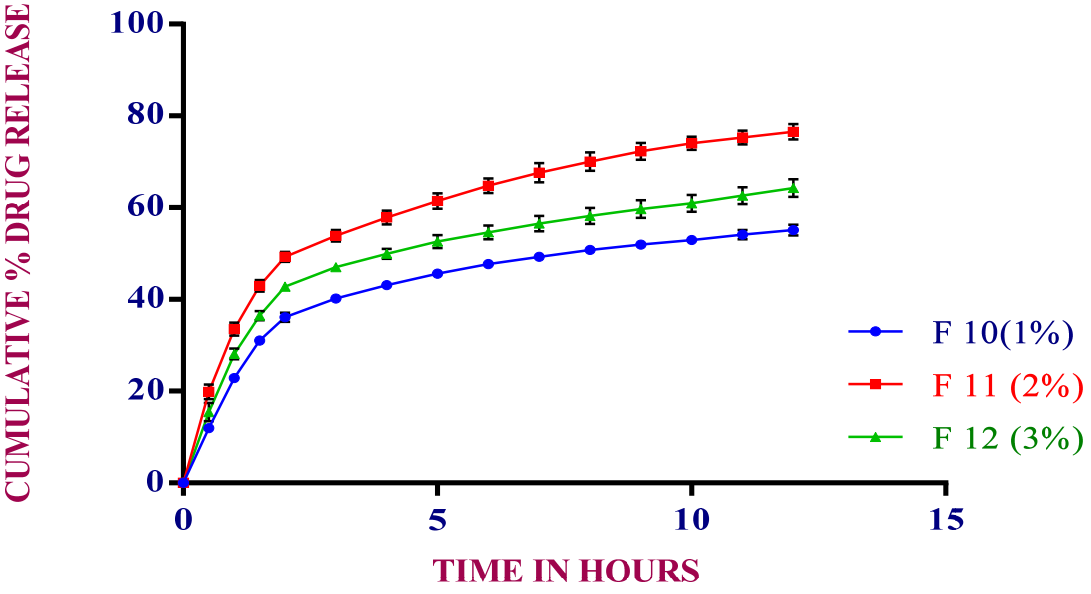


FIGURE 13(d).COMPARATIVE *INVITRO* DRUG RELEASE STUDY OF CEFAZOLIN LOADED BOVINE SERUM ALBUMIN NANOPARTICLES PREPARED AT 500 RPM IN pH 9.

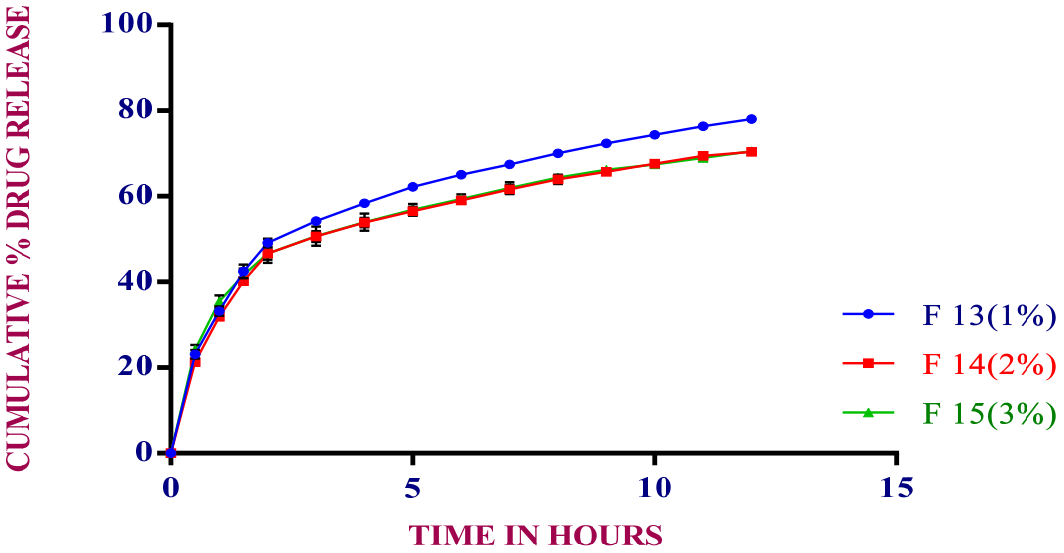


FIGURE 13(e).COMPARATIVE *INVITRO* DRUG RELEASE STUDY OF CEFAZOLIN LOADED BOVINE SERUM ALBUMIN NANOPARTICLES PREPARED AT 500 RPM IN pH 10.

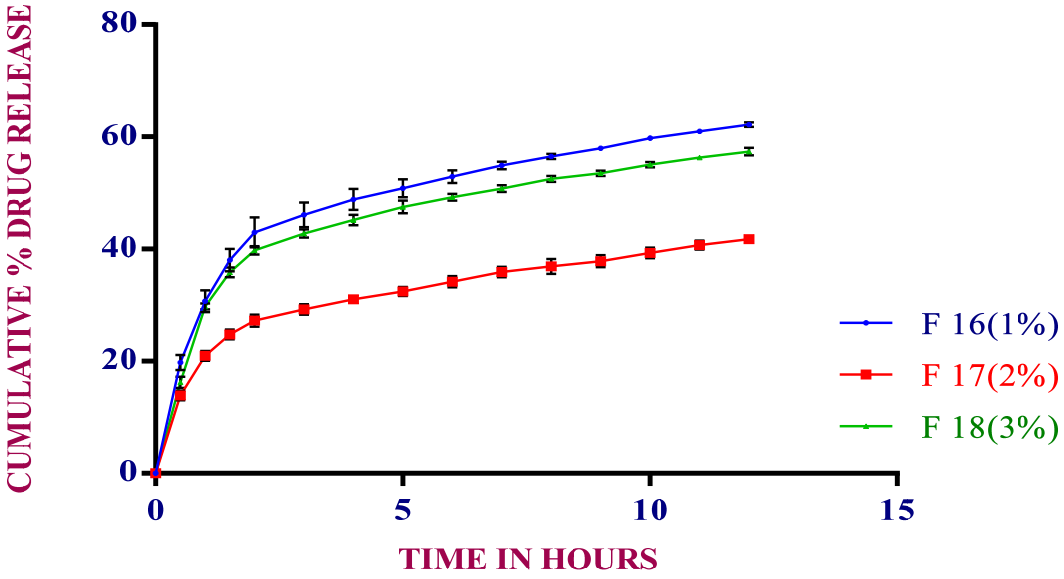


FIGURE 13(f).COMPARATIVE *INVITRO* DRUG RELEASE STUDY OF CEFAZOLIN LOADED BOVINE SERUM ALBUMIN NANOPARTICLES PREPARED AT 1000 RPM IN pH 8.

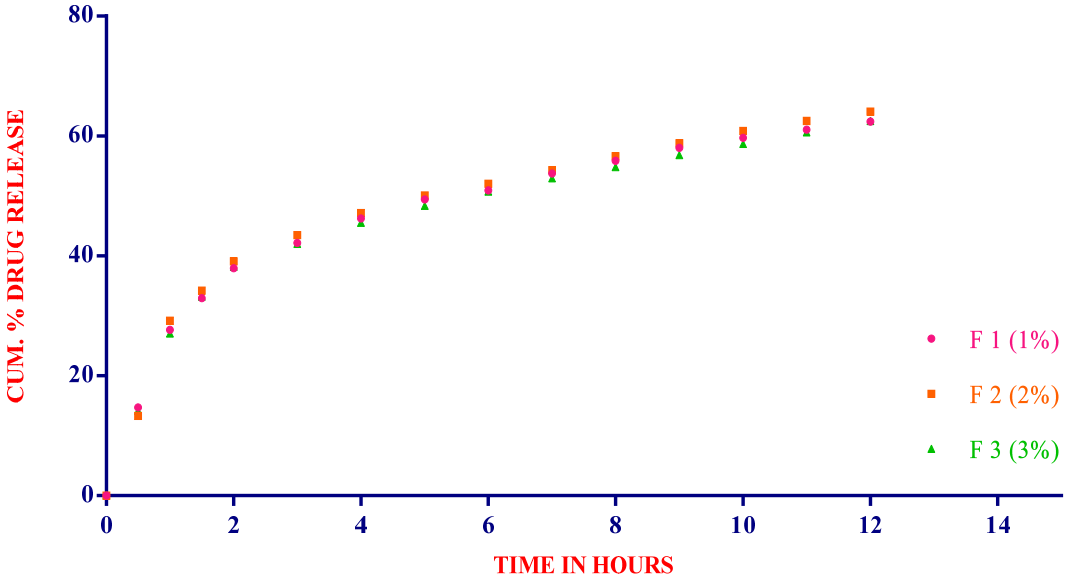


FIGURE 14(a). COMPARATIVE ZERO ORDER RELEASE KINETICS OF CEFAZOLIN LOADED BSA NANOPARTICLES, 1000 RPM, pH 9.

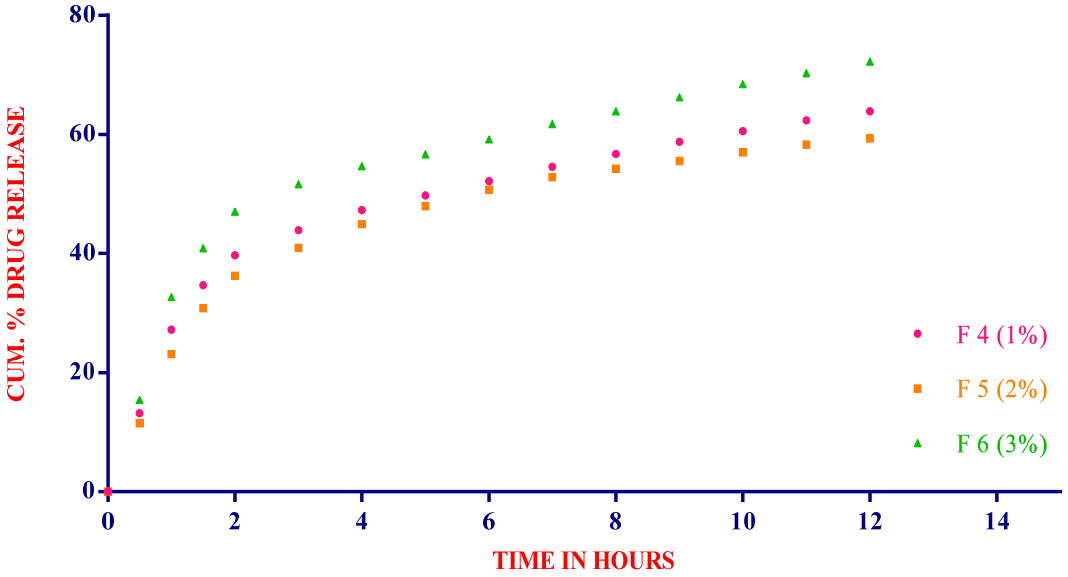


FIGURE 14(b). COMPARATIVE ZERO ORDER RELEASE KINETICS OF CEFAZOLIN LOADED BSA NANOPARTICLES, 1000 RPM, pH 10.

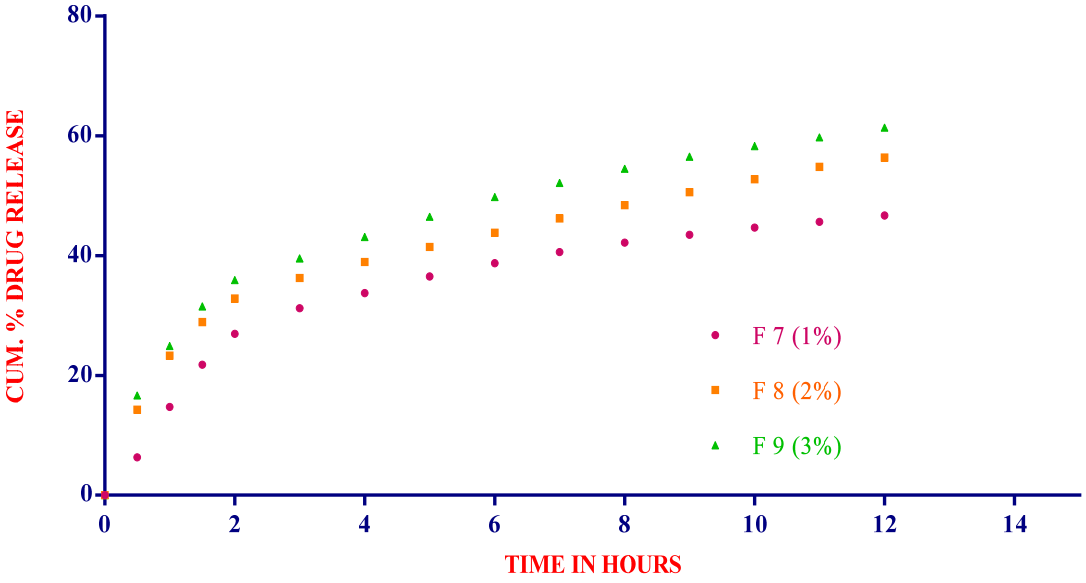


FIGURE 14(c). COMPARATIVE ZERO ORDER RELEASE KINETICS OF CEFAZOLIN LOADED BSA NANOPARTICLES, 1000 RPM, pH 8.

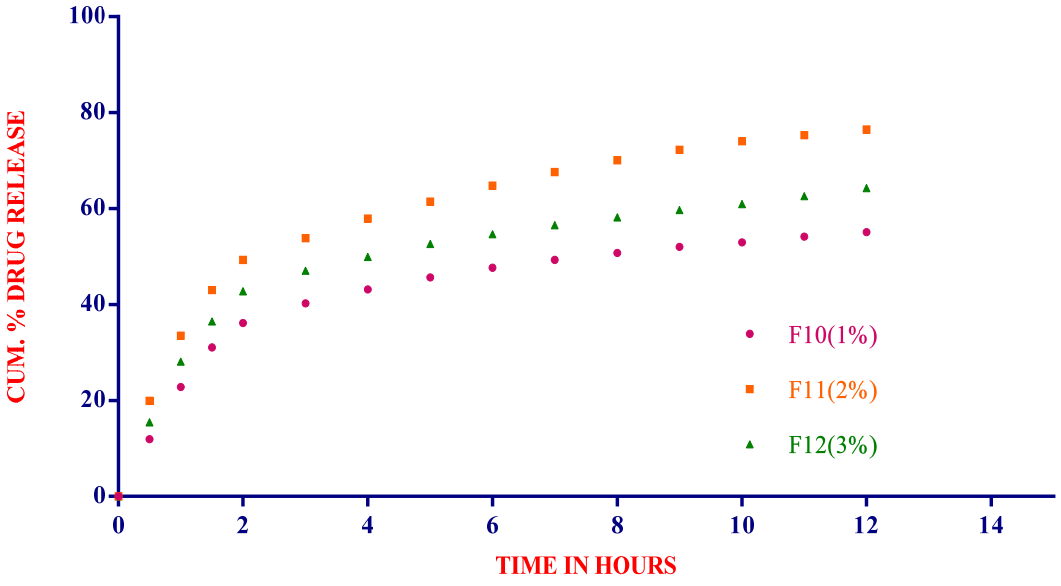


FIGURE 14(d). COMPARATIVE ZERO ORDER RELEASE KINETICS OF CEFAZOLIN LOADED BSA NANOPARTICLES, 500 RPM, pH 9.

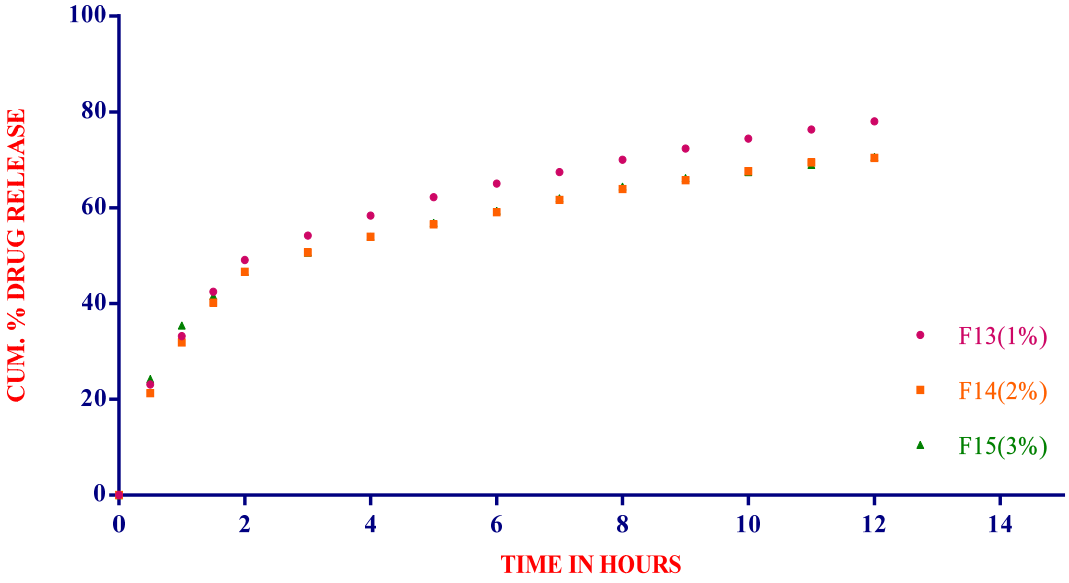


FIGURE 14(e). COMPARATIVE ZERO ORDER RELEASE KINETICS OF CEFAZOLIN LOADED BSA NANOPARTICLES, 500 RPM, pH 10.

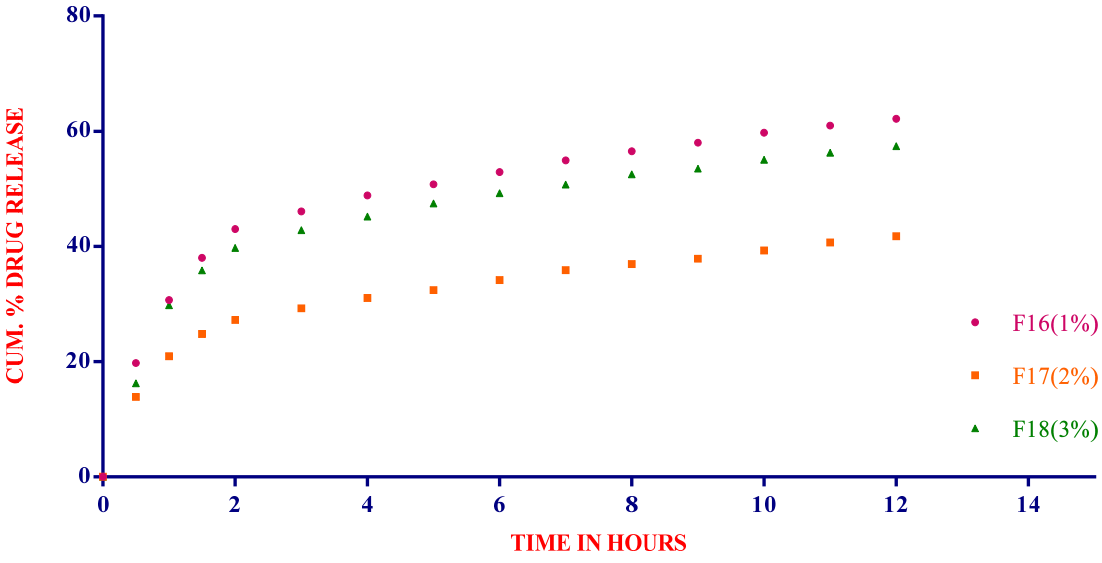


FIGURE 14(f). COMPARATIVE ZERO ORDER RELEASE KINETICS OF CEFAZOLIN LOADED BSA NANOPARTICLES, 500 RPM, pH 8.

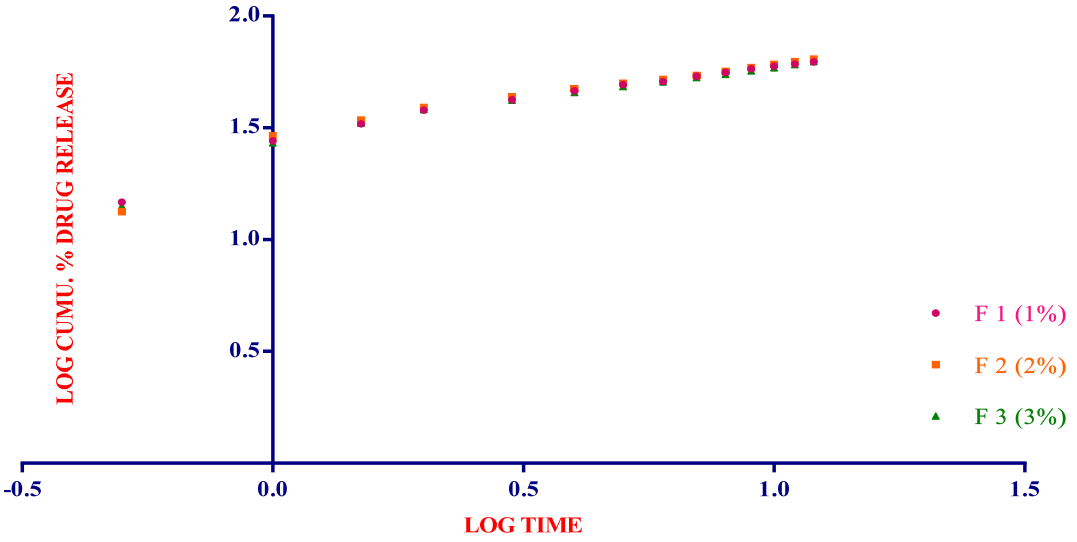


FIGURE 15(a). COMPARATIVE KORSEMEYER PEPPAS MODEL RELEASE KINETICS OF CEFAZOLIN LOADED BSA NANOPARTICLES, 1000 RPM, pH 9.

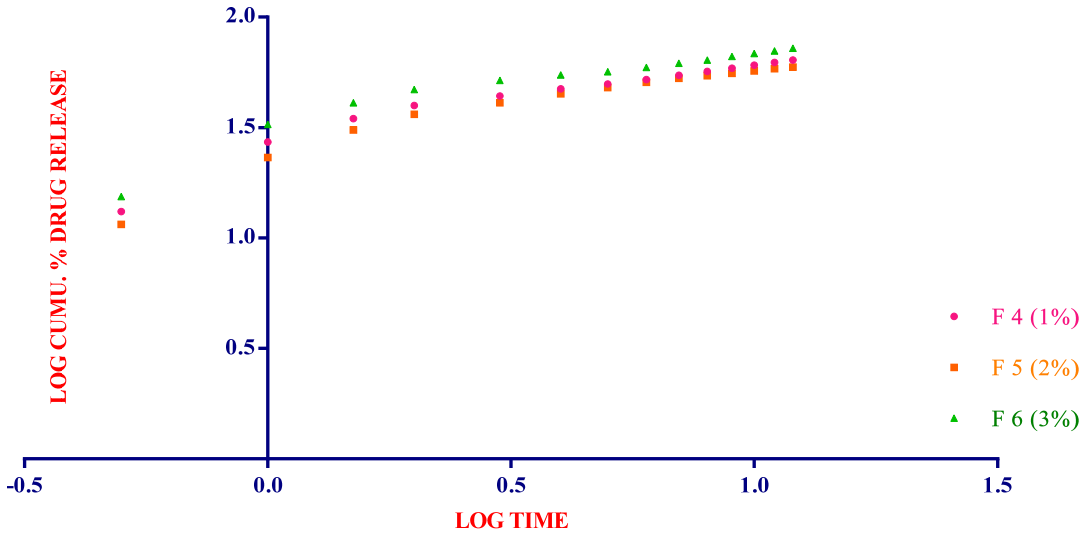


FIGURE 15(b).COMPARATIVE KORSEMEYER PEPPAS MODEL RELEASE KINETICS OF CEFAZOLIN LOADED BSA NANOPARTICLES, 1000 RPM, pH 10.

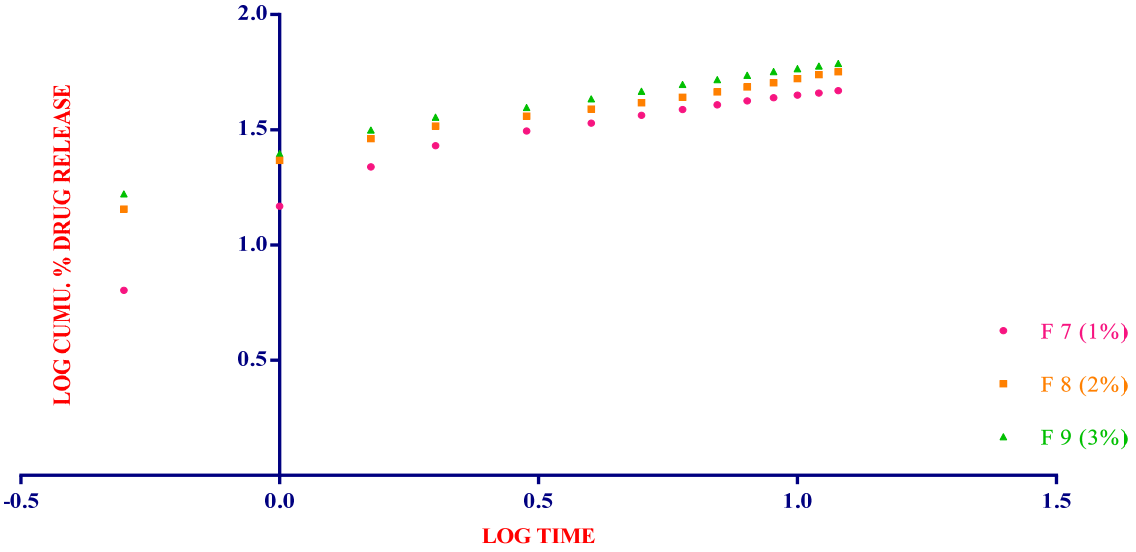


FIGURE 15(c). COMPARATIVE KORSEMEYER PEPPAS MODEL RELEASE KINETICS OF CEFAZOLIN LOADED BSA NANOPARTICLES, 1000 RPM, pH 8.

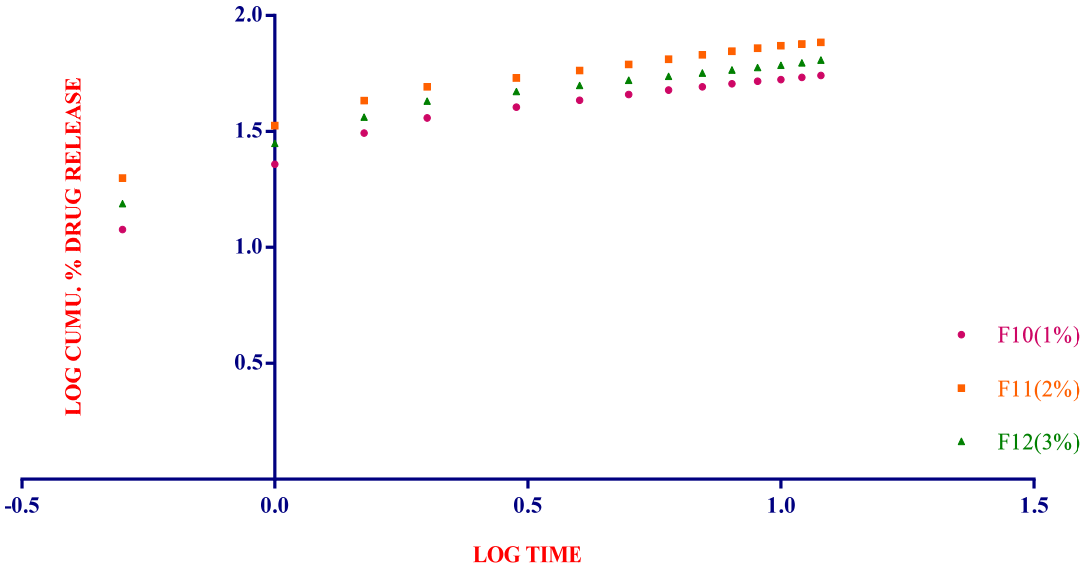


FIGURE 15(d).COMPARATIVE KORSEMEYER PEPPAS MODEL RELEASE KINETICS OF CEFAZOLIN LOADED BSA NANOPARTICLES, 500 RPM, pH 9.

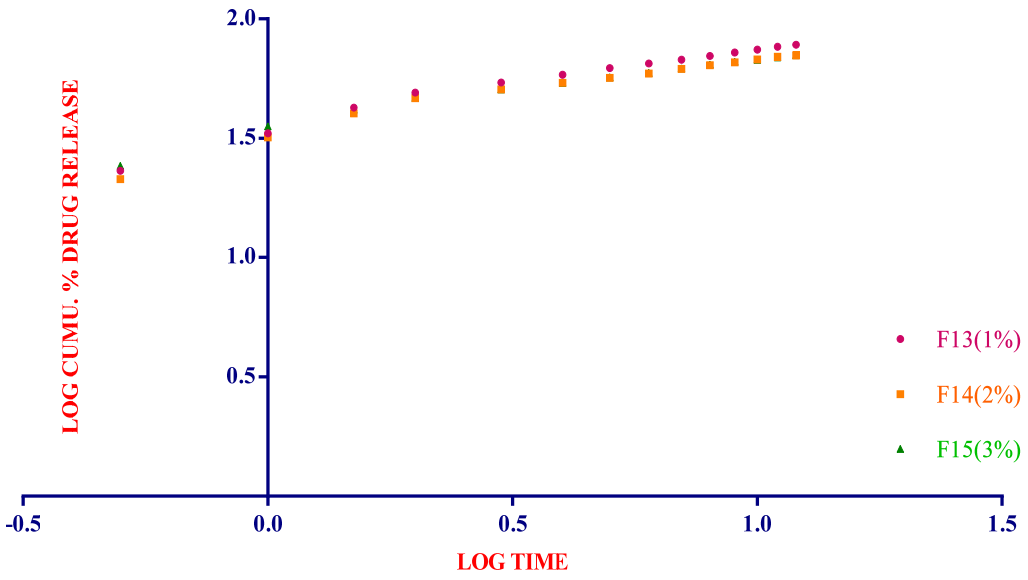


FIGURE 15(e). COMPARATIVE KORSEMEYER PEPPAS MODEL RELEASE KINETICS OF CEFAZOLIN LOADED BSA NANOPARTICLES, 500 RPM, pH 10.

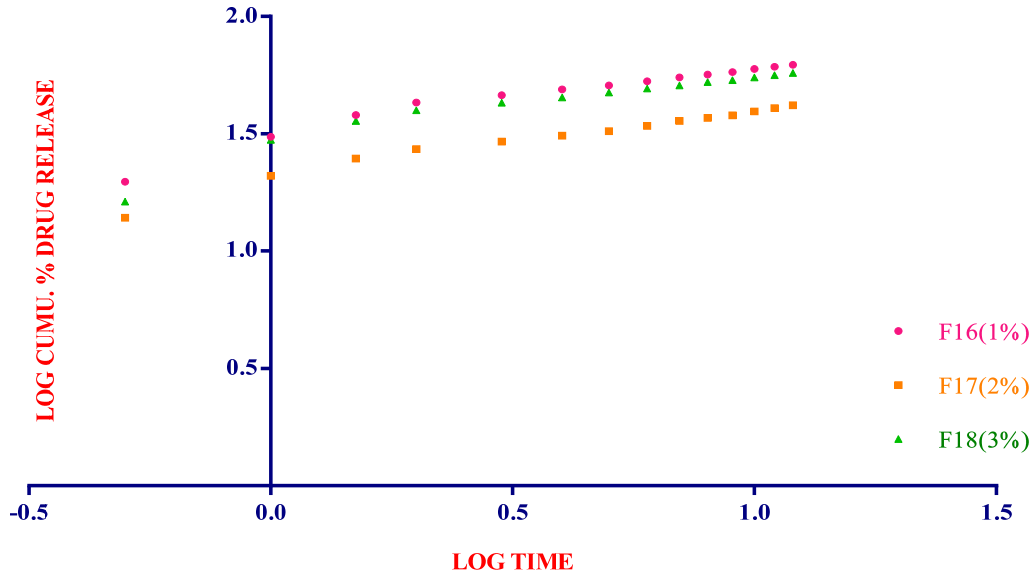


FIGURE 15(f). COMPARATIVE KORSEMEYER PEPPAS MODEL RELEASE KINETICS OF CEFAZOLIN LOADED BSA NANOPARTICLES, 500 RPM, pH 8.

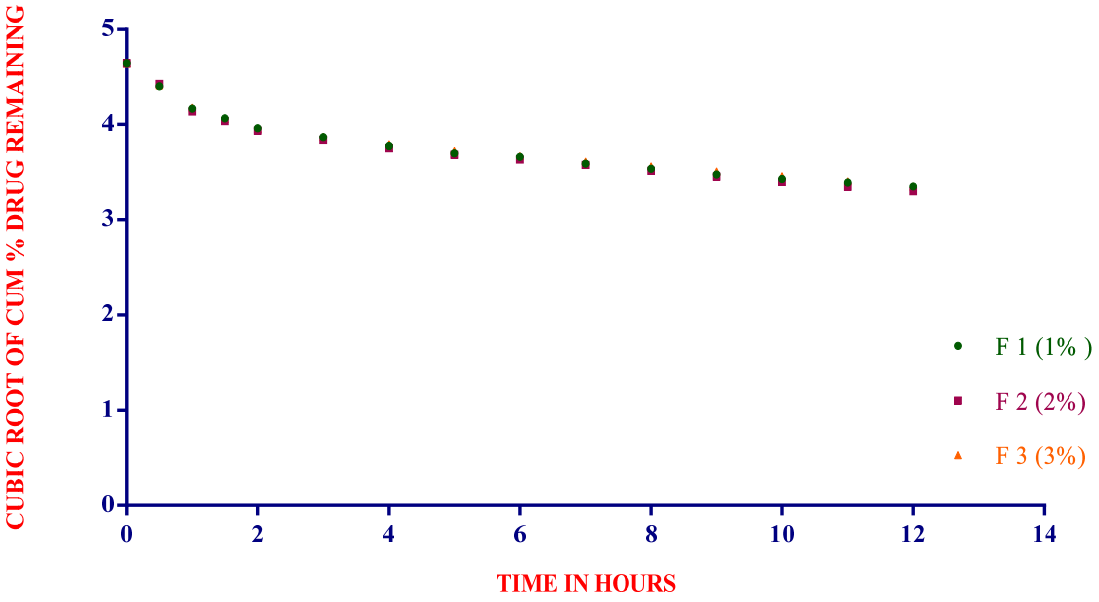


FIGURE 16(a). COMPARATIVE INVITRO HIXON CROWELL MODEL RELEASE KINETICS OF CEFAZOLIN LOADED BSA NANOPARTICLES, 1000 RPM, pH 9.

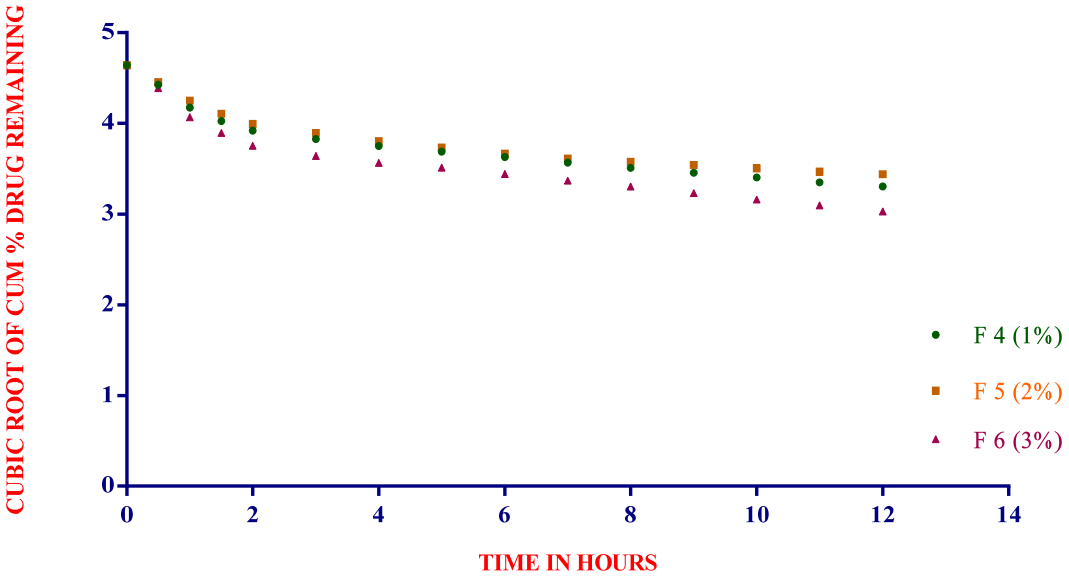


FIGURE 16(b). COMPARATIVE INVITRO HIXON CROWELL MODEL RELEASE KINETICS OF CEFAZOLIN LOADED BSA NANOPARTICLES, 1000 RPM, pH 10.

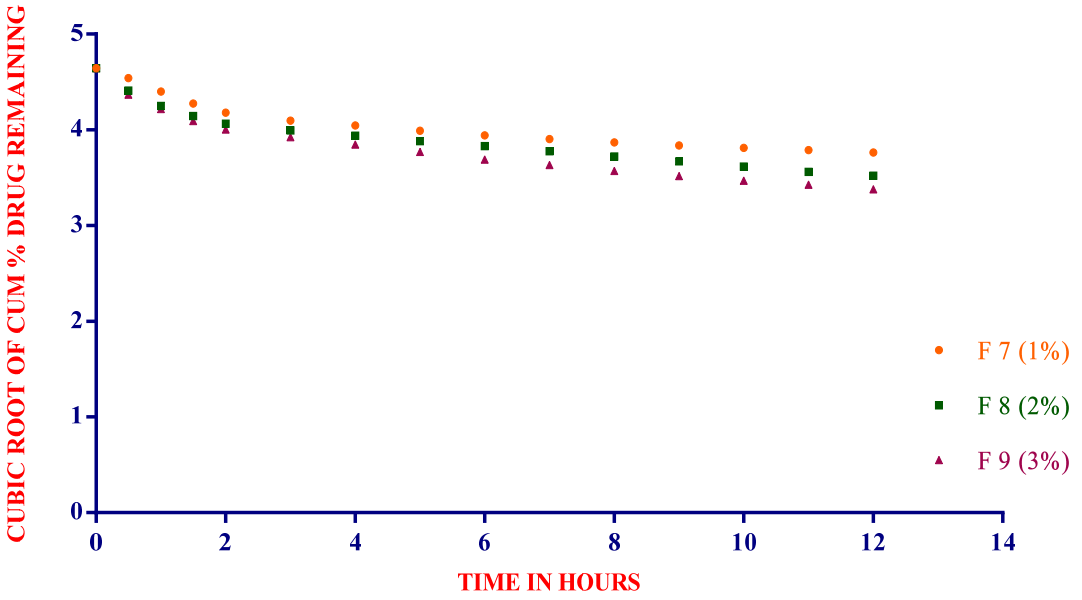


FIGURE 16(c). COMPARATIVE INVITRO HIXON CROWELL MODEL RELEASE KINETICS OF CEFAZOLIN LOADED BSA NANOPARTICLES, 1000 RPM, pH 8.

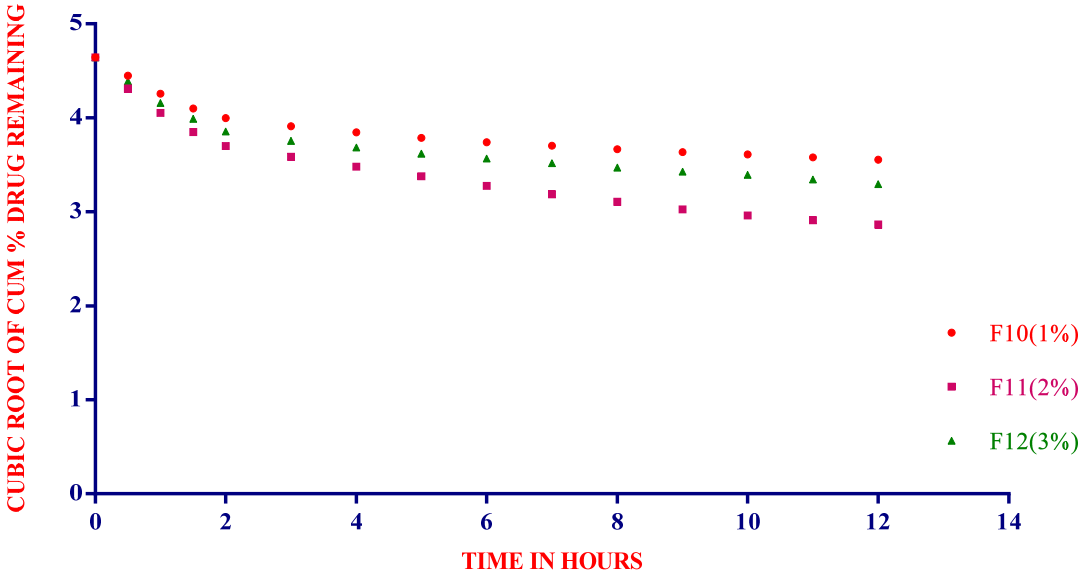


FIGURE 16(d). COMPARATIVE INVITRO HIXON CROWELL MODEL RELEASE KINETICS OF CEFAZOLIN LOADED BSA NANOPARTICLES, 500 RPM, pH 9.

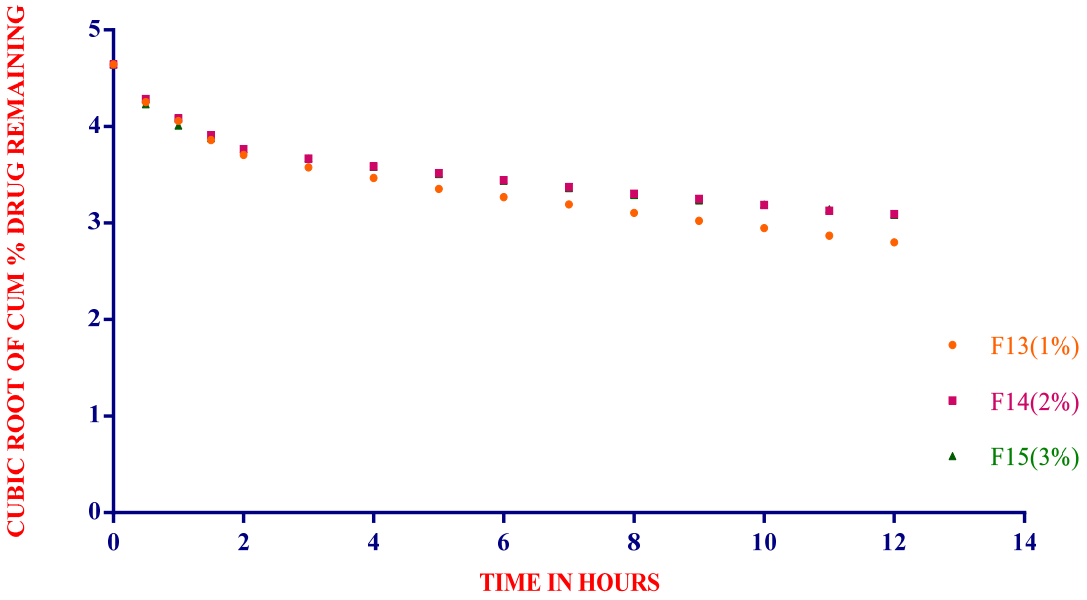


FIGURE 16(e). COMPARATIVE INVITRO HIXON CROWELL MODEL RELEASE KINETICS OF CEFAZOLIN LOADED BSA NANOPARTICLES, 500 RPM, pH 10.

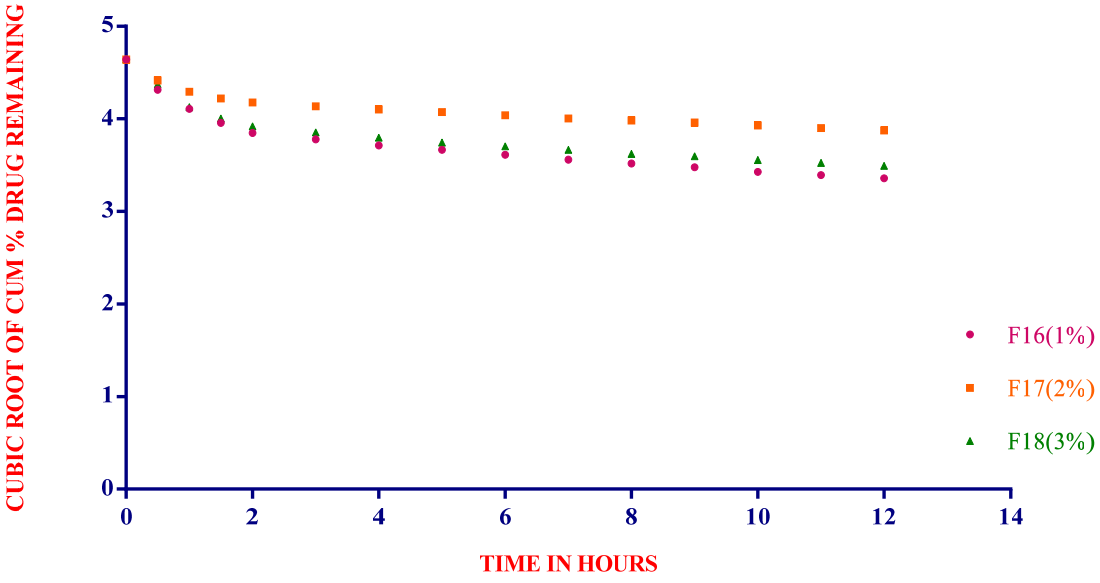


FIGURE 16(f).COMPARATIVE INVITRO HIXON CROWELL MODEL RELEASE KINETICS OF CEFAZOLIN LOADED BSA NANOPARTICLES, 500 RPM, pH 8.

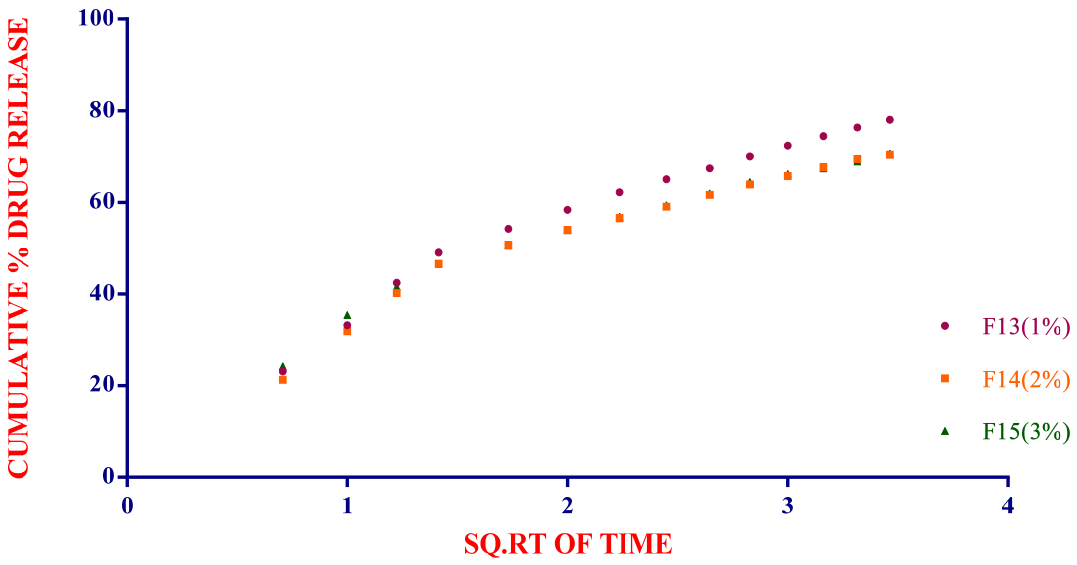


FIGURE 17(a). COMPARATIVE HIGUCHI MODEL RELEASE KINETICS OF CEFAZOLIN LOADED BSA NANOPARTICLES, 500 RPM, pH 10.

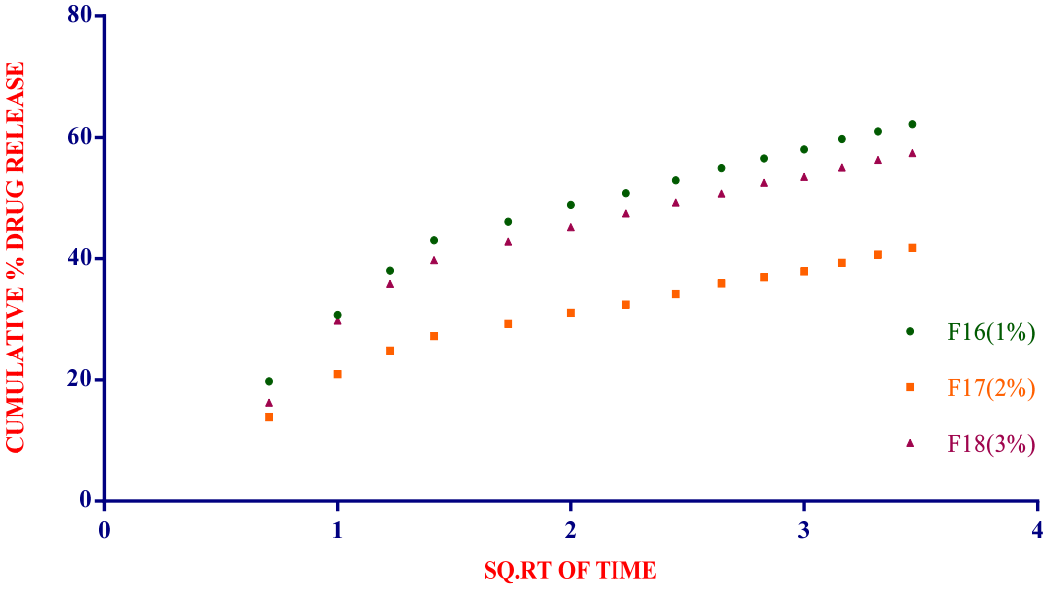


FIGURE 17(b). COMPARATIVE HIGUCHI MODEL RELEASE KINETICS OF CEFAZOLIN LOADED BSA NANOPARTICLES, 500 RPM, pH 8.

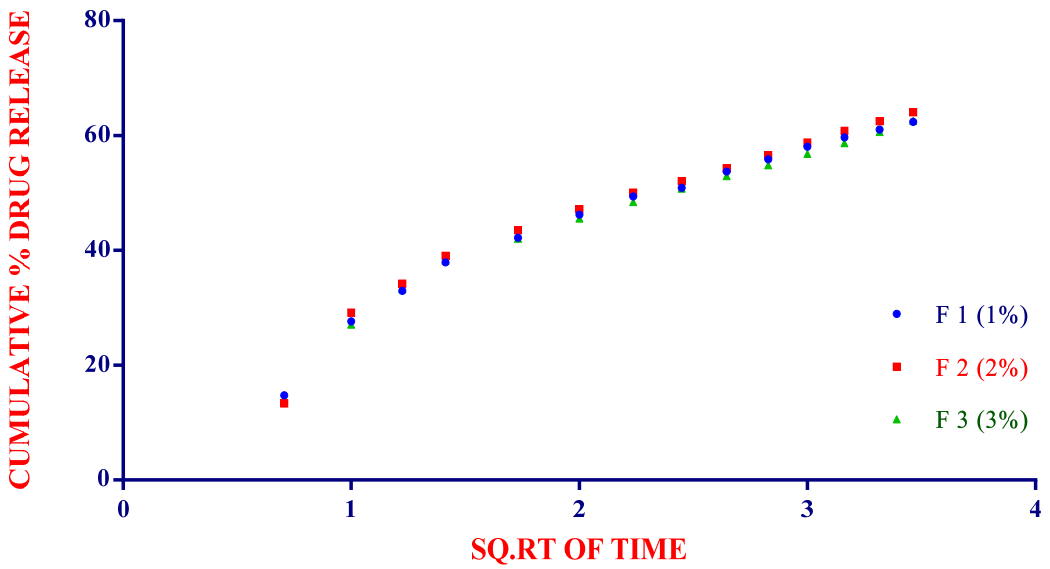


FIGURE 17(c). COMPARATIVE HIGUCHI MODEL RELEASE KINETICS OF CEFAZOLIN LOADED BSA NANOPARTICLES, 1000 RPM, pH 9.

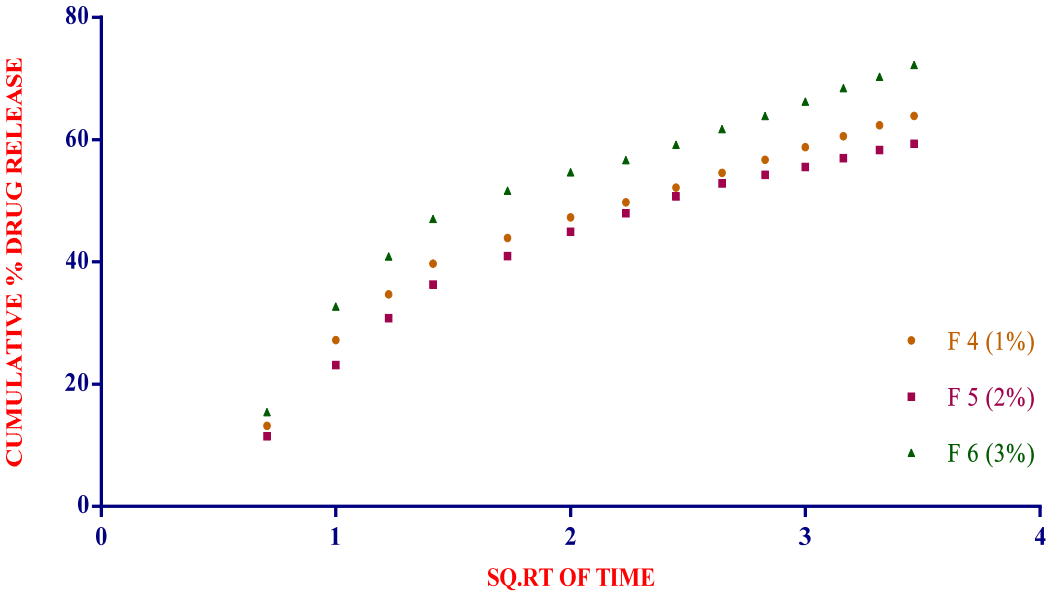


FIGURE 17(d).COMPARATIVE HIGUCHI MODEL RELEASE KINETICS OF CEFAZOLIN LOADED BSA NANOPARTICLES, 1000 RPM, pH 10.

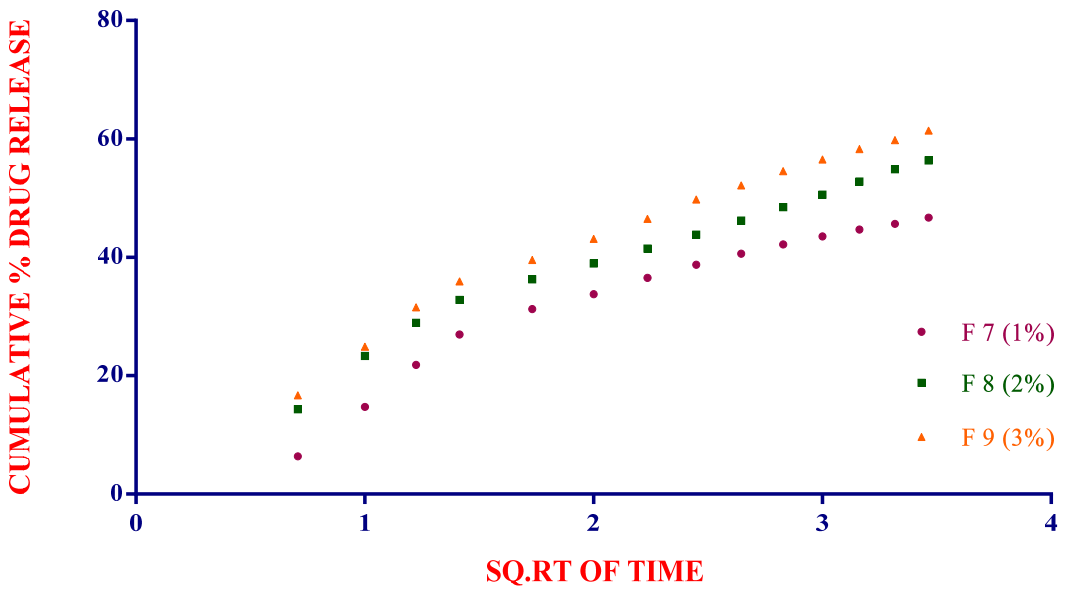


FIGURE 17(e). COMPARATIVE HIGUCHI MODEL RELEASE KINETICS OF CEFAZOLIN LOADED BSA NANOPARTICLES, 1000 RPM, pH 8.

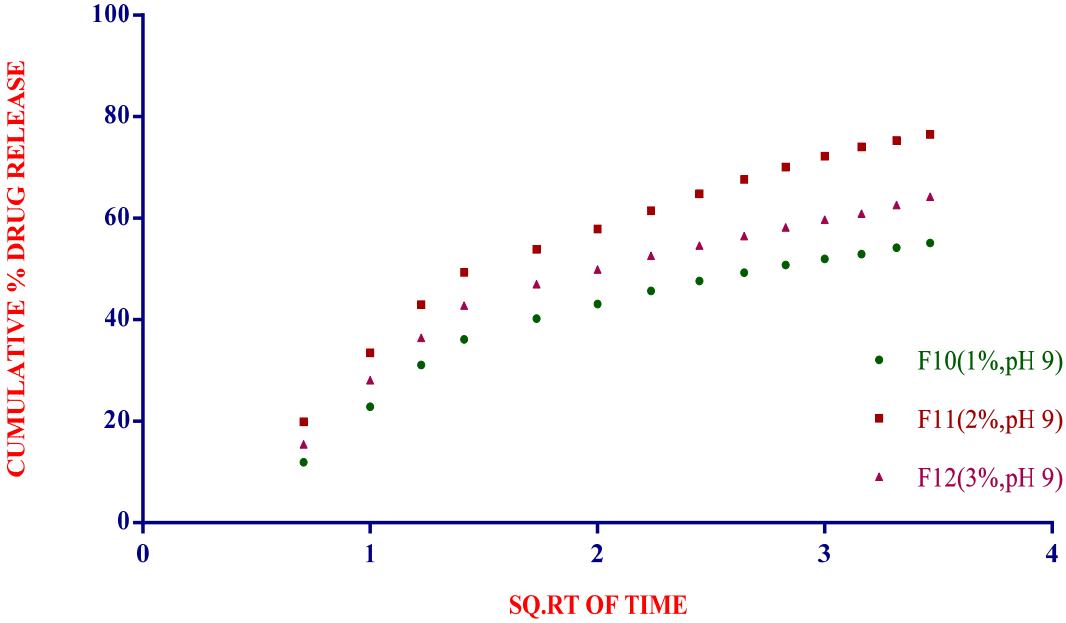


FIGURE 17(f). COMPARATIVE HIGUCHI MODEL RELEASE KINETICS OF CEFAZOLIN LOADED BSA NANOPARTICLES, 500 RPM, pH 9.

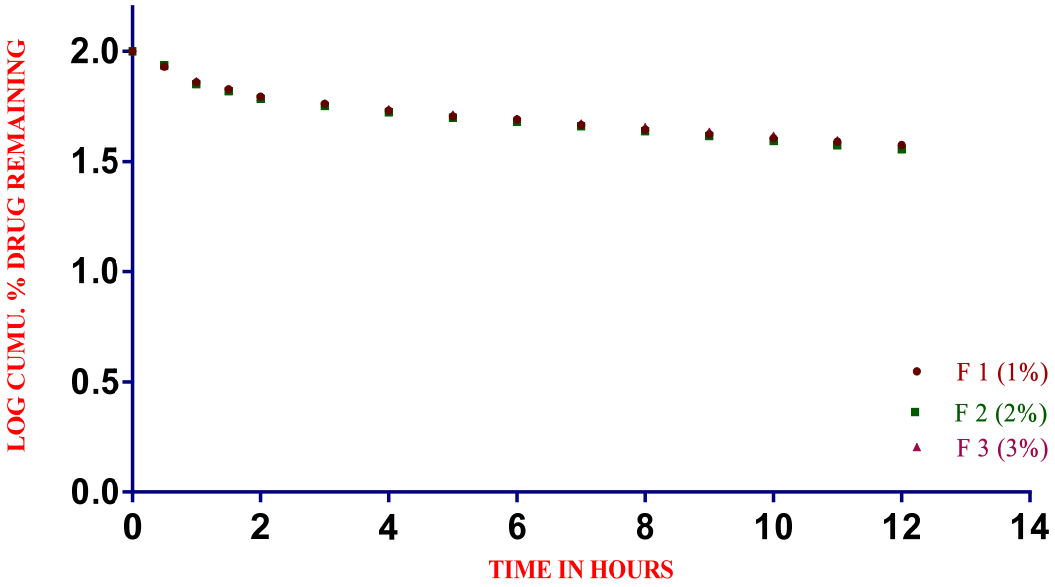


FIGURE 18(a). COMPARATIVE INVITRO FIRST ORDER RELEASE KINETICS OF CEFAZOLIN LOADED BSA NANOPARTICLES, 1000 RPM, pH 9.

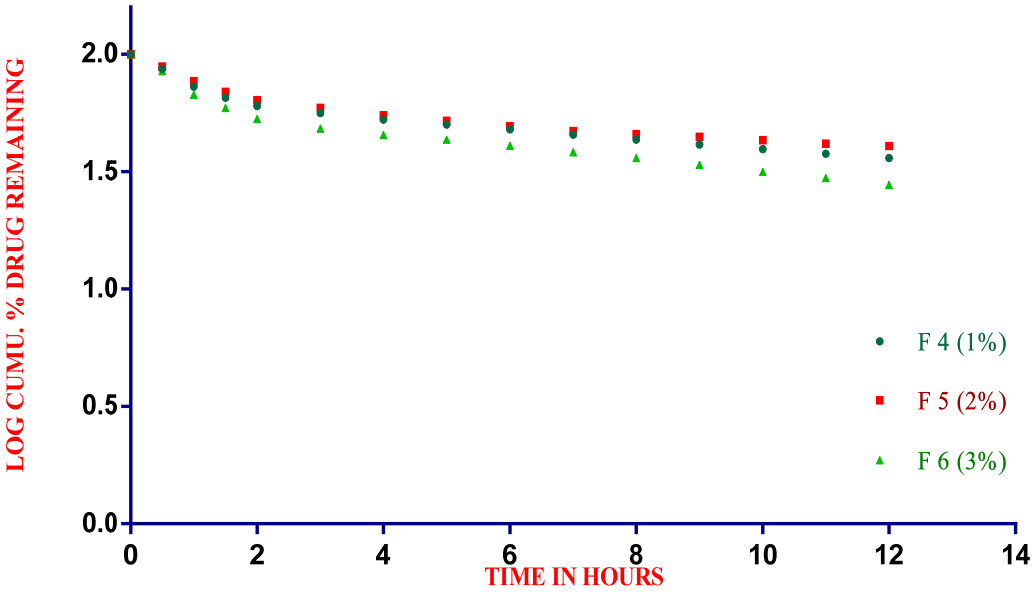


FIGURE 18(b). COMPARATIVE INVITRO FIRST ORDER RELEASE KINETICS OF CEFAZOLIN LOADED BSA NANOPARTICLES, 1000 RPM, pH 10.

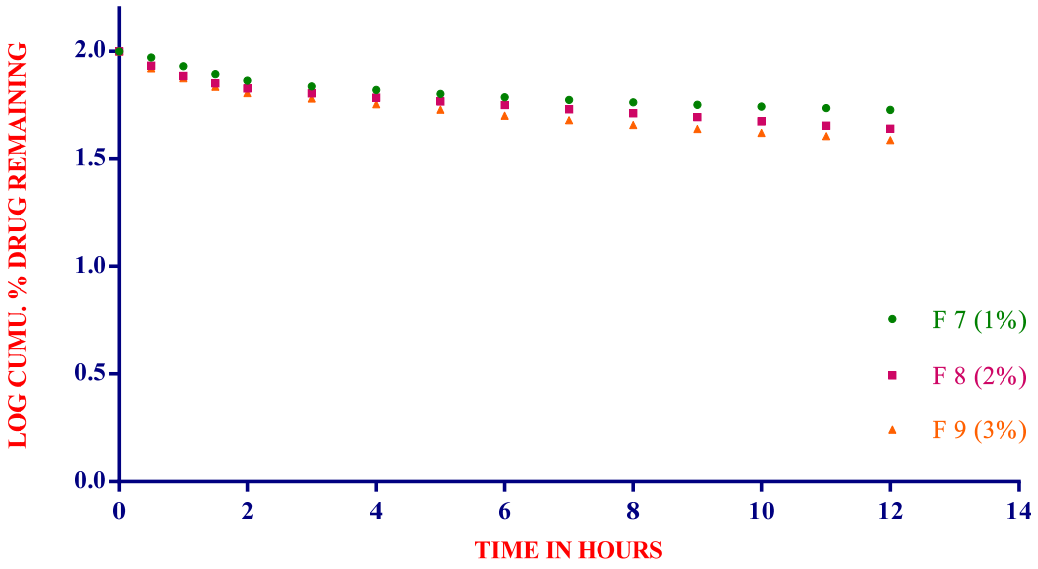


FIGURE 18(c).COMPARATIVE INVITRO FIRST ORDER RELEASE KINETICS OF CEFAZOLIN LOADED BSA NANOPARTICLES, 1000 RPM, pH 8.

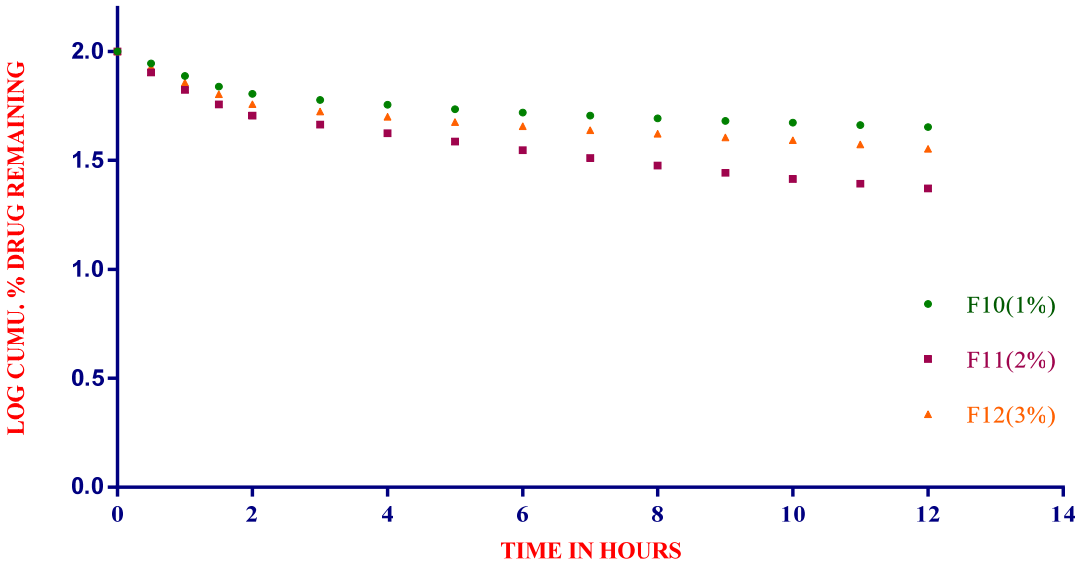


FIGURE 18(d). COMPARATIVE INVITRO FIRST ORDER RELEASE KINETICS OF CEFAZOLIN LOADED BSA NANOPARTICLES, 500 RPM, pH 9.

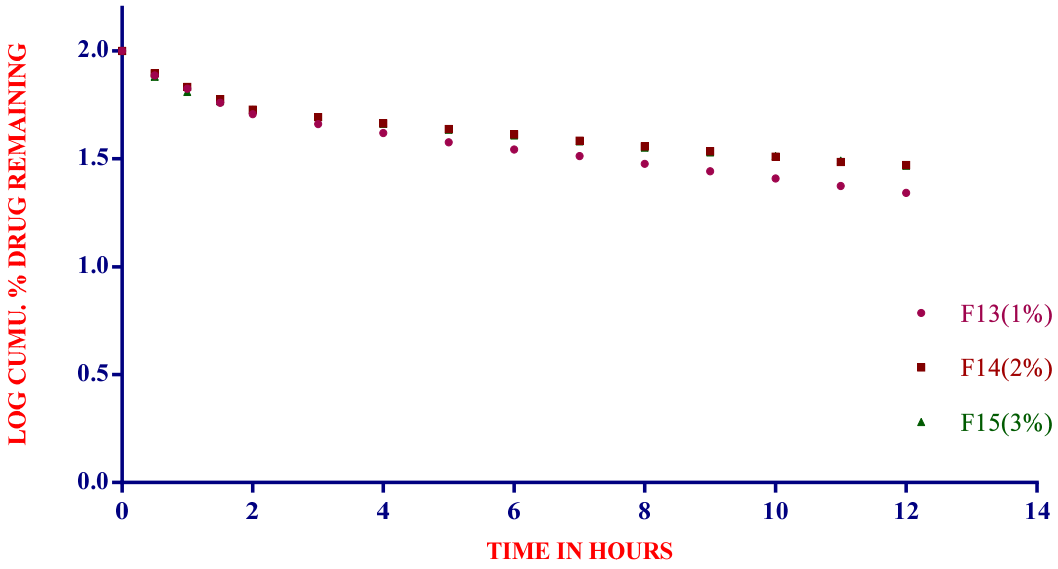


FIGURE 18(e). COMPARATIVE INVITRO FIRST ORDER RELEASE KINETICS OF CEFAZOLIN LOADED BSA NANOPARTICLES,500 RPM, pH 10.

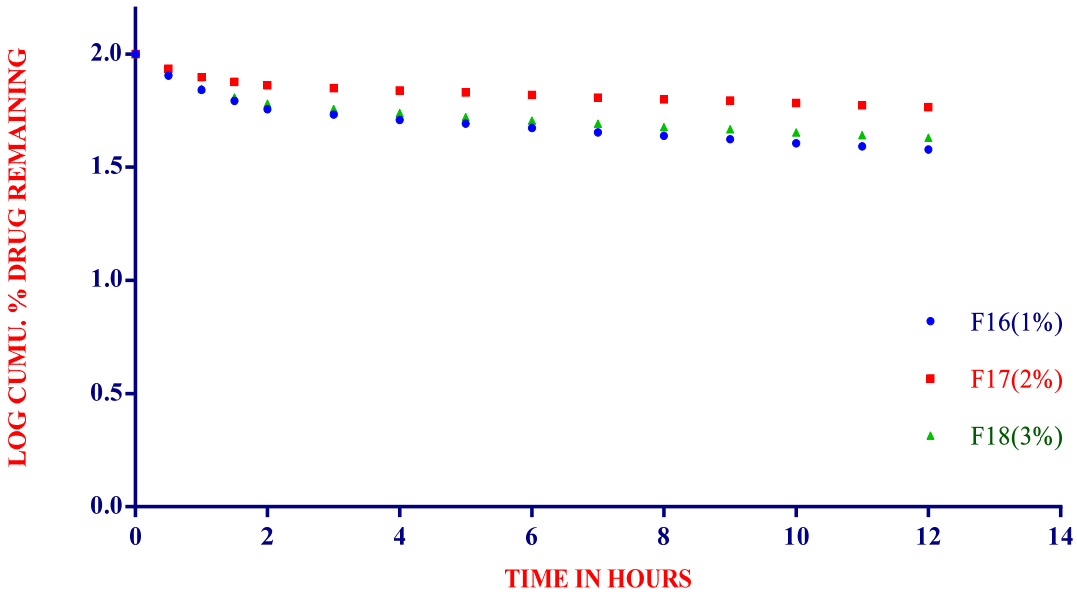


FIGURE 18(f). COMPARATIVE INVITRO FIRST ORDER RELEASE KINETICS OF CEFAZOLIN LOADED BSA NANOPARTICLES, 500 RPM, pH 8

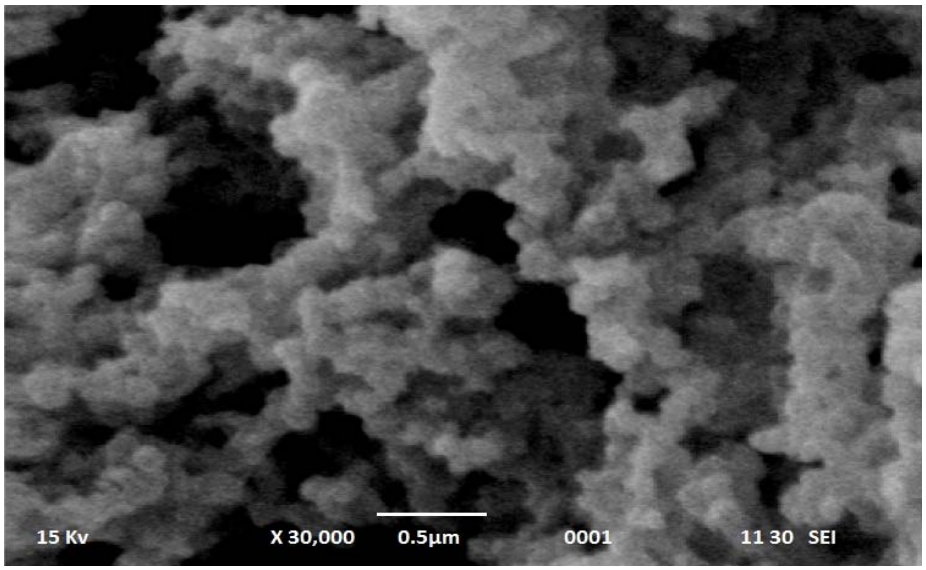


FIGURE 19. SCANNING ELECTRON MICROSCOPY OF CEFAZOLIN LOADED BSA NANOPARTICLES WITH CRYOPROTECTANT.

FIGURE 20. MICROBIOLOGICAL ASSAY OF CEFAZOLIN LOADED BSA NANOPARTICLES

S- STANDARD **1-CEF NP**

CHAPTER X

SUMMARY AND CONCLUSION

CHAPTER - X

SUMMARY AND CONCLUSION

Summary:

- In the present an attempt was made to develop nanoparticles of cefazolin with controlled particle size in order to increase its circulation half life thereby sustain its therapeutic activity using BSA as polymer.
- IR spectroscopy studies confirmed there was an interaction between drug and polymer.
- The cefazolin loaded nanoparticles were successfully prepared by desolvation technique using various concentration of BSA (1%, 2%, 3%) in different pH (8, 9, 10) at two different rpm (1000, 500).
- The drug content analysis showed minimum variations, suggesting uniform distribution of drug.
- The entrapment efficiency of all the formulations were decreased with increasing pH and not affected by polymer concentration.
- Particle size analyzer (Malvern) used to explore the particle size of cefazolin loaded BSA nanoparticles showed a particle size in the range of 46.9nm-345.3nm.
- The polydispersity index of nanoparticle formulations F1-F18 was less than 0.5 except F16(0.608), which indicated a relatively homogenous dispersion of particles.
- Zeta potential of cefazolin BSA nanoparticles showed a negative surface charge due to the presence of carboxylic groups in the polymer.
- The invitro drug release studies displayed a biphasic drug release pattern with a burst release within 0.5 hrs followed by sustained release for 12hours.

- Invitro drug release kinetics showed sustained release and Fickian diffusion mechanism which indicated that the drug release was diffusion controlled.
- On the basis of particle size and release data formulation F7 (prepared using 1% BSA + pH 8 + 1000 rpm) showed a good sustained release profile with high entrapment efficiency.
- SEM images confirmed the morphology of the drug loaded nanoparticles (F7) was sphere and had particle size less than 100nm.
- The infra red spectrum of nanoparticle formulation shows changes in amino group functional peak, it indicates the nanoparticle crosslinked by glutaraldehyde.
- Microbiological assay revealed that the nanoparticle formulation retained their antibacterial activity and also showed zone of inhibition 36mm after 24 hours.

Conclusion:

Hence, Cefazolin loaded albumin nanoparticles can be successfully prepared by coacervation following crosslinking with glutaraldehyde. The characterizations of the resulting nanoparticles were dependent on the polymer concentration, pH and the rpmof thestirrer during the desolvating process. And number of variables which might affect the final nanoparticle size was investigated to optimize the formulation.The preparation process of BSA nanoparticles was optimized by varying various parameter pH(8-10),polymer concentration (1%-3%) and rpm of the stirrer (1000,500), wherein pH 8, 1% polymer concentration and 1000rpm were found to be most favorable. The invitro release studies indicated there was an initial burst release of the drug in 0.5 hours followed by sustained release for 12 hours. According to the literature, the results demonstrated that produced nanoparticles have sufficient properties as a carrier for drug delivery systems.

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